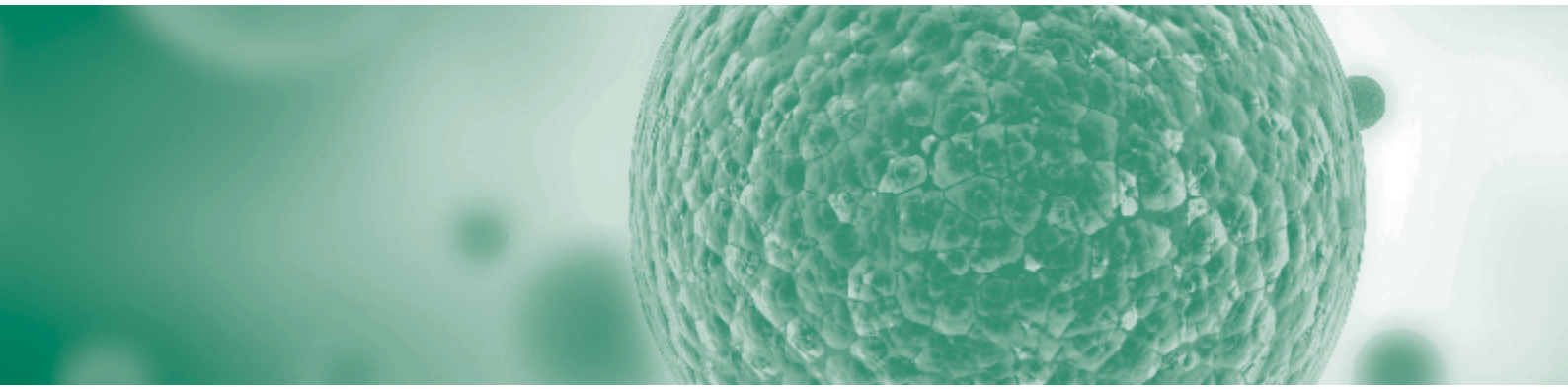
CELLESTIAL® ASSAY KITS & DYES



Cell Death & Viability

Autophagy
Aggresome & Inclusion Body Detection
Apoptosis/Necrosis
Senescence
Viability

Cell Function

Cell Cycle Analysis
Cell Tracking & Lineage
Calcium Mobilization
Oxidative Stress
Hypoxia
Multidrug Resistance

Cell Structure & Organelle Detection

Nucleus
Endoplasmic Reticulum
Golgi Apparatus
Lysosomes
Mitochondria
Multiple Organelles

Gold Standard Dyes

Common Fluorescent Probes for Cellular Analysis

Global Research, Global Reach.™

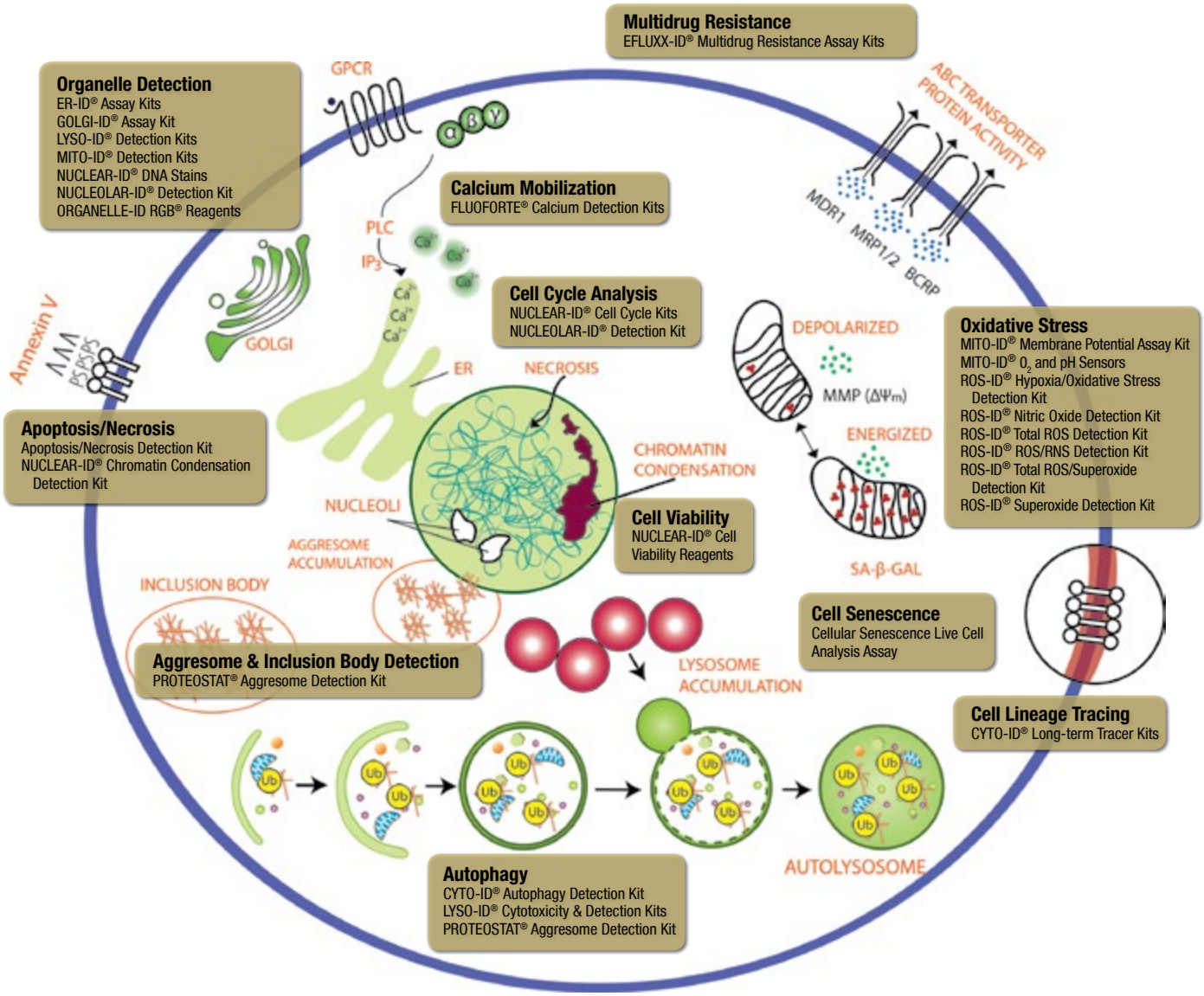
CONTENTS

VISUALIZE CELLULAR RESPONSES WITH CELLESTIAL® FLUORESCENT PROBES

Our CELLESTIAL® portfolio of fluorescent probes and assay kits for cellular analysis provides a complete set of tools for monitoring cell viability, proliferation, death, oxidative stress and toxicology by flow cytometry, microscopy and microplate platforms. Our assays and probes have been optimized for the most demanding imaging applications including confocal microscopy, wide-field fluorescence microscopy, flow cytometry, and high content screening where consistency and reproducibility are essential.

Our photostable fluorescent probes are developed to maximize compatibility with common counterstains and fluorescent marker-expressing cell lines. CELLESTIAL® probes allow pharmaceutical and biotech researchers to accelerate their drug development programs through early lead compound identification, lead candidate selection, predictive toxicology and compound characterization.

More than 300 assay kits and over 3,000 highly characterized antibodies support further dissection of these responses. Custom formulation and bulk production of antibodies are also available to fit your specific needs.


Multidrug Resistance
EFLUXX-ID® Multidrug Resistance Assay Kits
Organelle Detection
ER-ID® Assay Kits
GOLGI-ID® Assay Kit
LYSO-ID® Detection Kits
MITO-ID® Detection Kits
NUCLEAR-ID® DNA Stains
NUCLEOLAR-ID® Detection Kit
ORGANELLE-ID RGB® Reagents
GPCR
ABC TRANSPORTER PROTEIN ACTIVITY
MDR1 MRP1/2 BCRP
Calcium Mobilization
FLUOFORTE® Calcium Detection Kits
PLC
IP3
Cell Cycle Analysis
NUCLEAR-ID® Cell Cycle Kits
NUCLEOLAR-ID® Detection Kit
GOLGI
Annexin V
PS PS PS
ER
NECROSIS
DEPOLARIZED
MMP (ΔΨm)
ENERGIZED
Oxidative Stress
MITO-ID® Membrane Potential Assay Kit
MITO-ID® O2 and pH Sensors
ROS-ID® Hypoxia/Oxidative Stress Detection Kit
ROS-ID® Nitric Oxide Detection Kit
ROS-ID® Total ROS Detection Kit
ROS-ID® ROS/RNS Detection Kit
ROS-ID® Total ROS/Superoxide Detection Kit
ROS-ID® Superoxide Detection Kit
Apoptosis/Necrosis
Apoptosis/Necrosis Detection Kit
NUCLEAR-ID® Chromatin Condensation Detection Kit
CHROMATIN CONDENSATION
NUCLEOLI
Cell Viability
NUCLEAR-ID® Cell Viability Reagents
AGGRESOME ACCUMULATION
INCLUSION BODY
SA-β-GAL
Cell Senescence
Cellular Senescence Live Cell Analysis Assay
Aggresome & Inclusion Body Detection
PROTEOSTAT® Aggresome Detection Kit
LYSOSOME ACCUMULATION
Cell Lineage Tracing
CYTO-ID® Long-term Tracer Kits
Ub
AUTOLYSOSOME
Autophagy
CYTO-ID® Autophagy Detection Kit
LYSO-ID® Cytotoxicity & Detection Kits
PROTEOSTAT® Aggresome Detection Kit

CELL DEATH & VIABILITY

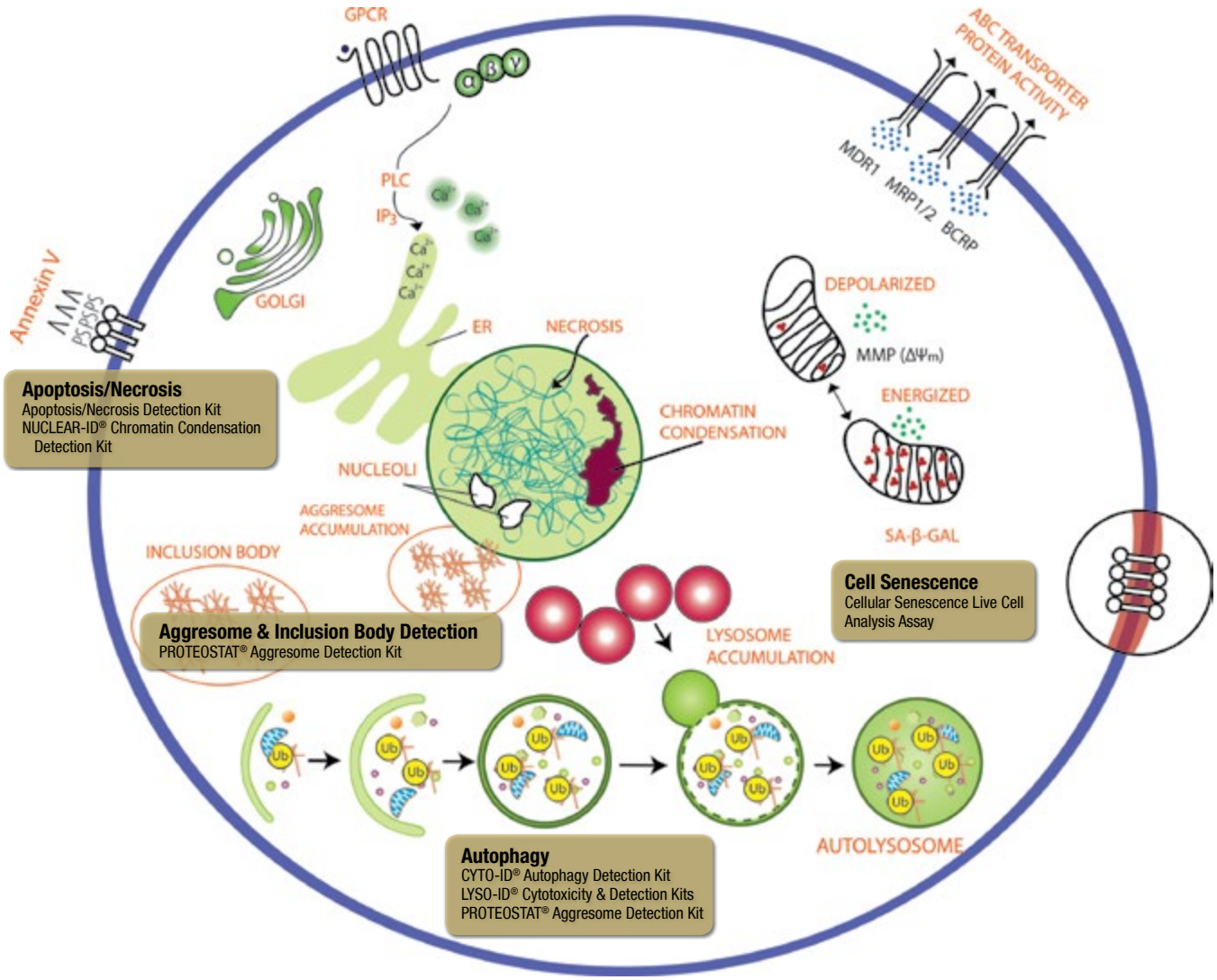
Many categories of cell death exist, but perhaps the most studied and well-recognized are the 3 major classes of programmed cell death (PCD) highlighted by the Nomenclature Committee on Cell Death (NCCD) – Autophagy, Apoptosis, and Necrosis.

Apoptosis (PCD type 1) is the best-characterized type of programmed cell death because of its importance in development and homeostasis, and in the pathogenesis of different diseases such as cancer. Apoptotic cells die in a controlled fashion in response to a variety of extrinsic or intrinsic signals (e.g., activation of TNF receptors, DNA damage, mitochondrial pathways). The hallmarks of apoptotic cell death include exposure of phosphatidylserine on the extracellular face of the plasma membrane, activation of caspases, disruption of mitochondrial membrane potential, cell shrinkage, DNA fragmentation and DNA condensation.

Autophagy (PCD type 2) is selective degradation of intracellular targets, such as misfolded proteins and damaged organelles, and is an important homeostatic function. Autophagy performs in concert with the ubiquitin-proteasome system (UPS) to degrade aggregated/misfolded proteins that are ubiquitinylated, targeting them for degradation by autophagy. The ubiquitinylated cargo is carried to the phagophore and surrounds its cargo, forming a double-membrane vesicle, the autophagosome. Then, the lysosome fuses to the autophagosome and the cargo is degraded inside the autolysosome.

Necrosis (PCD type 3) is an uncontrolled cell death characterized by cell swelling, as well as destruction of the plasma membrane and subcellular organelles, without nuclear fragmentation and condensation. Necrotic cell death is considered a heterogeneous phenomenon including both programmed and accidental cell death. Necrosis is often defined in a negative manner, as a type of cell death that involves rupture of the plasma membrane without the hallmarks of apoptosis and without massive autophagic vacuolization.

Enzo Life Sciences offers a wide range of novel products for researching and analyzing cell death. Our product line includes over 1,000 different antibodies, reagents and assay kits spanning all 3 main areas of cell death – autophagy, apoptosis and necrosis.



Apoptosis/Necrosis
Apoptosis/Necrosis Detection Kit
NUCLEAR-ID® Chromatin Condensation Detection Kit

Cell Senescence
Cellular Senescence Live Cell Analysis Assay

Aggresome & Inclusion Body Detection
PROTEOSTAT® Aggresome Detection Kit

Autophagy
CYTO-ID® Autophagy Detection Kit
LYSO-ID® Cytotoxicity & Detection Kits
PROTEOSTAT® Aggresome Detection Kit

AUTOPHAGY

CYTO-ID® AUTOPHAGY DETECTION KIT (10662-266 / 89165-926)

A no-transfection quantitative assay for monitoring autophagy in live cells

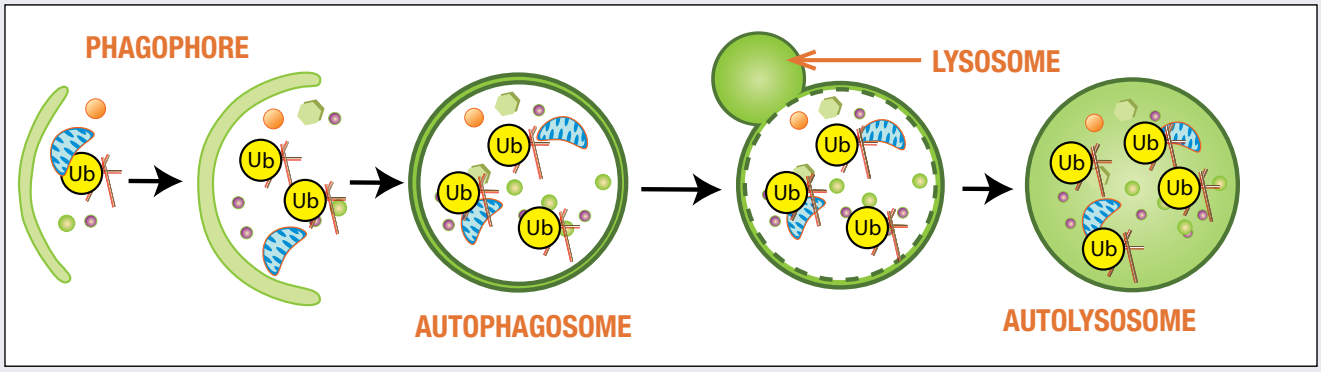
Autophagy is a stress-induced protective mechanism. Less active under basal conditions, the mechanism is utilized by eukaryotic cells through lysosome-mediated bulk degradation of cellular contents when subjected to certain hostile conditions such as nutrient depletion and chemical or environmental stress. The role of increased autophagic activity in the pathology of cancer, neurodegeneration, cardiovascular disease and diabetes has become widely recognized and commonly studied.

CYTO-ID® Autophagy Detection Kit measures autophagic vacuoles and monitors autophagic flux in live cells using a novel dye that selectively labels autophagic vacuoles. The dye has been optimized through the identification of titratable functional moieties that allow for minimal staining of lysosomes while exhibiting bright fluorescence upon incorporation into pre-autophagosomes, autophagosomes, and autolysosomes (autophagolysosomes). The assay offers a rapid and quantitative approach to monitoring autophagy in live cells without the need for cell transfection.

- No transfection required
- Proprietary dye includes titratable moietes specific for selectively staining autophagic vesicles
- Protocol validated with known inhibitors and activators of autophagic activity
- Rapidly quantifies autophagy in native heterogeneous cell populations
- Eliminates need for time and effort-consuming transfection efficiency validation required with LC3-GFP transfection
- Selective and comprehensive staining, allows measurement and differentiation between autophagic flux and autophagolysosome accumulation
- Negligible staining of lysosomes reduces background seen with other dyes
- Facilitates high-throughput screening of activators and inhibitors of autophagy

Mechanism of Action & Autophagy Pathway

The probe is a cationic amphiphilic tracer (CAT) dye that rapidly partitions into cells in a similar manner as drugs that induce phospholipidosis. Careful selection of titratable functional moieties on the dye prevent its accumulation within lysosomes, but enables labeling of vacuoles associated with the autophagy pathway.



Autophagy is selective degradation of intracellular targets, such as misfolded proteins and damaged organelles, and is an important homeostatic function. Autophagy performs in concert with the ubiquitin-proteasome system (UPS) to degrade aggregated/misfolded proteins that are ubiquitinylated, targeting them for degradation by autophagy. The ubiquitinylated cargo is carried to the phagophore and surrounds its cargo, forming a double membrane vesicle, the autophagosome. Then, the lysosome fuses to the autophagosome and the cargo is degraded inside the autolysosome.

AUTOPHAGY

Profile Autophagy Without Transfection

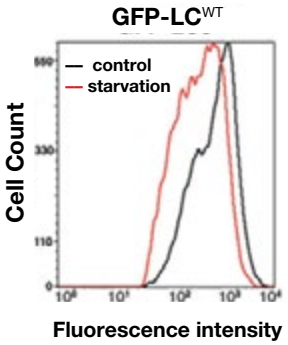


Figure 1A: CHO cells stably expressing GFP-LC3 transfected cell lines results in relatively poor baseline separation of control-vs-starved cell populations, making quantification of autophagy difficult. Figure adapted from Shvets E, Fass E, Elazar Z.

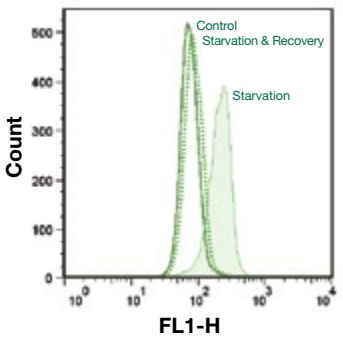


Figure 1B: The CYTO-ID® Autophagy Detection Kit specifically labels autophagic vacuoles independent of LC3 protein and eliminates the need for transfection. HeLa cells were subjected to starvation and recovery and then labeled with CYTO-ID® Green detection reagent. The dye enables clear detection and quantification of autophagic and pre-autophagic vacuoles that directly correlates to induction of autophagy.

Visualization of Autophagic Accumulation and Autophagic Flux

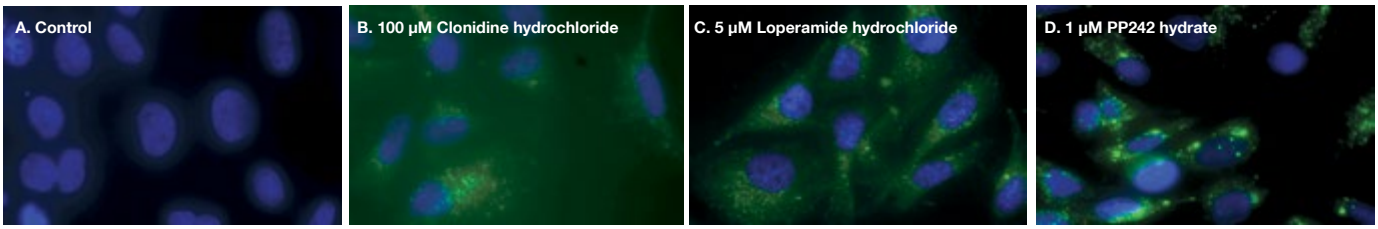


Figure 2: Autophagic vacuole accumulation and flux are both detected by CYTO-ID® Autophagy Green dye as observed by fluorescence microscopy. HeLa cells were mock-induced with 0.2% DMSO (A) or induced with 100 µM Clonidine hydrochloride (B), 5 µM Loperamide hydrochloride (C) or 1 µM PP242 hydrate (D) for 12 hours at 37°C. After treatment, cells were incubated with CYTO-ID® Green Detection Reagent for 10 min at 37°C and then washed with assay buffer. Nuclei were counter-stained in blue with Hoechst 33342 dye.

Description	Cat. No.	Kit Components	
CYTO-ID® Autophagy Detection Kit	10662-266 / 89165-926	Dyes, Rapamycin, Chloroquine, 10x assay buffer	
CYTO-ID® Autophagy Detection Kit 2.0	75802-912 / 75802-914	Dyes, Rapamycin, Chloroquine, 10x assay buffer	
Dyes Included in Kit	**Excitation/Emission**	**Platform**	**Cell Type**
CYTO-ID® Green Detection Reagent Hoechst 33342 Nuclear Stain	463/538nm ● 350/461nm ●		Live

Citations

1. S. Guo, et al. "A rapid and high content assay that measures cyto-ID-stained autophagic compartments and estimates autophagy flux with potential clinical applications." Autophagy. (2015) 11(3) 560-72.
2. S. Lisanti, et al. "Deletion of the Mitochondrial Chaperone TRAP-1 Uncovers Global Reprogramming of Metabolic Networks." Cell Rep. (2014) 8(3) 671-677.
3. Zhou Z.W., et al. "Induction of apoptosis and autophagy via sirtuin1- and PI3K/Akt/mTOR-mediated pathways by plumbagin in human prostate cancer cells." Drug Des. Devel. Ther. (2015) 9:1511.
4. Clarke A J., Ellinghaus U., Cortini A., Stranks A., Simon A K., Botto M., Vyse T.J. "Autophagy is activated in systemic lupus erythematosus and required for plasmablast development." Ann Rheum Dis. (2015) 74(5):912-20.
5. Weis S.N., Toniazzo A.P., Ander B P., Zhan X., Careaga M., Ashwood P., Wyse A.T., Netto C.A., Sharp F.R. "Autophagy in the brain of neonates following hypoxia-ischemia shows sex- and region-specific effects." Neuroscience. (2014) 256:201-9.
6. Shiroto T., Romero N., Sugiyama T., Sartoretto J.L., Kalwa H., Yan Z., Shimokawa H., Michel T. "Caveolin-1 is a critical determinant of autophagy, metabolic switching, and oxidative stress in vascular endothelium." PLoS One. (2014)9(2):e87871.
7. Xu M., Li X.X., Chen Y., Pitzer A.L., Zhang Y., Li P.L. "Enhancement of dynein-mediated autophagosome trafficking and autophagy maturation by ROS in mouse coronary arterial myocytes." J Cell Mol Med. (2014)18(11):2165-75.

Application Notes

1. Response Profiles of Known Autophagy-Modulators Across Multiple Cell Lines: Using CYTO-ID® Autophagy Dye to assess Compound Activity and Toxicity.
2. Cell-Based Screening of Focused Bioactive Compound Libraries: Assessing Small Molecule Modulators of the Canonical Wnt Signaling and Autophagy-Lysosome Pathways.
3. A Novel Image-Based Cytometry Method for Autophagy Detection in Living Cells.
4. Predictive High-Content/High-Throughput Assays for Hepatotoxicity Using Induced Pluripotent Stem Cell (iPSC)-Derived Hepatocytes.
5. Visualizing subcellular vesicles to quantitate autophagy in neuronal cells.

AUTOPHAGY

LYSO-ID® RED CYTOTOXICITY KIT (GFP-CERTIFIED®) (89165-892)

A rapid, quantitative and HTS-compatible live cell cytotoxicity assay

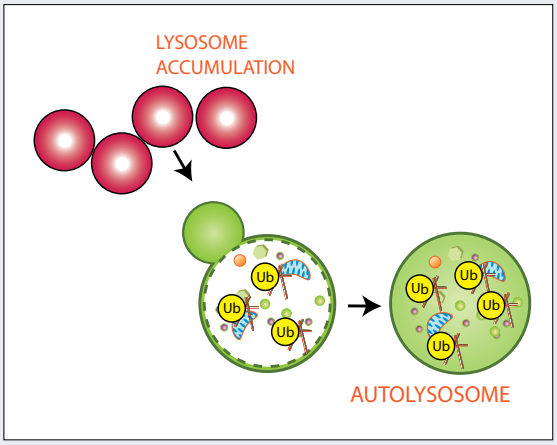
A hallmark of cellular toxicity in response to cationic amphiphilic drugs (CADs) and basic compounds is the onset of phospholipidosis, the formation and accumulation of large acidic vacuoles (i.e. lamellar bodies or lysosomal inclusion bodies) within the cell due to impaired degradation of lysosomes.

The LYSO-ID® Red Cytotoxicity Kit monitors dysfunction of lysosomal degradation using a drug-l ke cationic amphiphilic tracer (CAT) dye that rapidly and selectively stains acidic organelles, and is suitable for monitoring accumulation of lysosomes and lysosome-like structures in live cells.

- Assay includes unique drug-like dye that rapidly partitions into cells and labels acidic organelles
- Only commercial assay available that allows for long-term cell monitoring of cytotoxic effects
- Multi-well, high-throughput with rapid 10-15 minute dye incubation
- No co-incubation with artificial phospholipid analogs required for detection, eliminating the potential for confounding dye-associated artifacts
- Monitors lysosome accumulation as a response to prolonged drug treatment
- Quantitative results in as little as 3 hours, including drug treatment

Mechanism of Action

A cationic amphiphilic tracer (CAT) dye that rapidly partitions into cells in a similar manner as drugs that induce phospholipidosis. This tracer was created by placing titratable groups on the probe (dye), to enable labeling to be expanded into lamellar inclusion bodies of cells pretreated with weakly basic cell-permeant compounds, such as the antimalarial drug chloroquine. Besides lysosomes themselves, the probe can be employed for highlighting lysosome-like organelles under conditions wherein cells produce vacuoles that contain most of the degradative enzymes of the lysosome but are not as acidic as the parent organelle.



Eliminate Confounding Dye-associated Artifacts

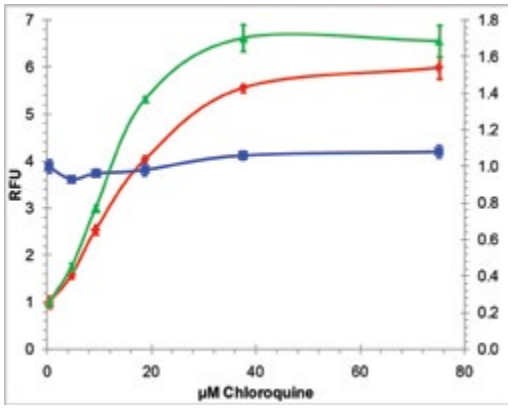


Figure 1: The short 15-minute LYSO-ID® Red dye incubation eliminates the potential for confounding dye-associated artifacts. Relative fluorescent intensity of U-2 OS cells treated with chloroquine at different concentrations for 24 h. Cells stained with LipidTox™ dye (green line) were incubated in the presence of the fluorescent lipid for 24 h during treatment with the drugs. Cells stained with LYSO-ID® Red dye (red line) or Hoechst 33342 (blue line) were stained for 15 min after drug incubation.

AUTOPHAGY

High Throughput Screening of Therapeutics for Lysosome-perturbing Activity

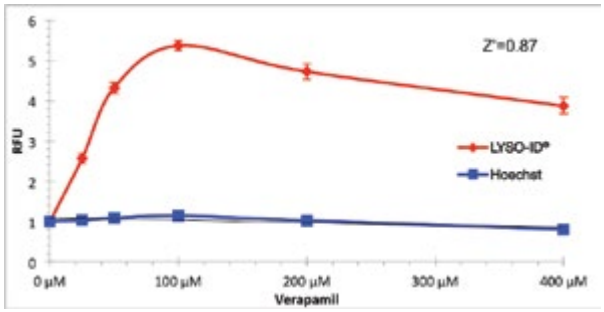


Figure 2: Toxicity of Verapamil in U-2 OS cells was estimated using a conventional fluorescence microplate reader. U-2 OS cells were treated with Verapamil for 18 hours and stained with LYSO-ID® Red dye for 15 minutes. The high Z-factor (0.87 for 100 µM Verapamil) indicates that LYSO-ID® Red dye is suitable for HTS applications. Hoechst is used as a counterstain as a normalization control for cell number.

Monitor Abnormal Lysosome Accumulation Arising from Drug-induced Phospholipidosis

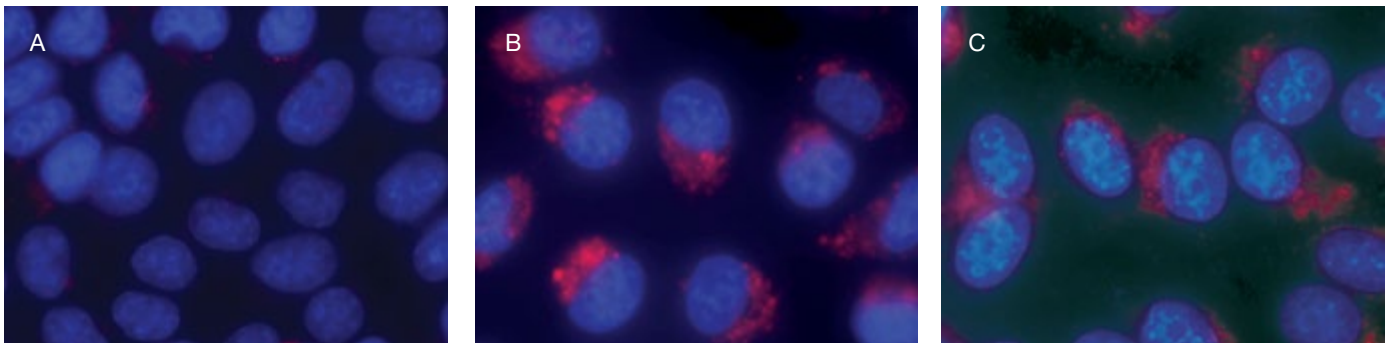


Figure 3: Drug-induced lysosome accumulation in U-2 OS cells was evaluated using LYSO-ID® Red dye. (A) Untreated cells (B) Chlorpromazine, 28 µM (C) Verapamil, 200 µM. These two compounds are cationic and amphiphilic, and known to induce abnormal accumulation of phospholipids within lysosomes, resulting in lamellar bodies. Treatment of U-2 OS cells with phospholipidosis-inducing drugs causes an increase in lysosome number and volume, detected as an increase in red fluorescence. Nuclei are counter-stained with Hoechst 33342 dye (blue).

Description	Cat. No.	Kit Components	
LYSO-ID® Red Cytotoxicity Kit	89165-892	10x dual color detection reagent, (mixture of dyes), detection buffer, Verapamil, 10x assay buffer	
Dyes Included in Kit	**Excitation/Emission**	**Platform**	**Cell Type**
LYSO-ID® Red Detection Reagent Hoechst 33342 Nuclear Stain	568/667nm ● 350/461nm ●		Live

Citations

1. François A., Terro F., Janet T., Rioux Bilan A., Paccalin M., Page G. "Involvement of interleukin-1 in the autophagic process of microglia: relevance to Alzheimer's disease." Neuroinflammation. (2013) 10:151.
2. Coleman J., Xiang Y., Pande P., Shen D., Gatica D., and Patton W. "A live-cell fluorescence microplate assay suitable for monitoring vacuolation arising from drug or toxic agent treatment." J Biomol Screen. (2010) 15(4):398-405.
3. Held, P., Newick, K., Shen, D. and Patton W. "Automated Detection of Drug-Induced Lysosomal Cytotoxicity - Automation of the Lyso-ID Red Assay Using the EL406 Combination Washer Dispenser". Lab Manager Magazine (June, 2010).

Application Note

1. Cell-Based Screening of Focused Bioactive Compound Libraries: Assessing Small Molecule Modulators of the Canonical Wnt Signaling and Autophagy-Lysosome Pathways.

AGGRESOME & INCLUSION BODY DETECTION

PROTEOSTAT® AGGRESOME DETECTION KIT (89409-190 / 10662-314)

Robust, quantitative detection of aggresomes relevant to the study of neurodegenerative disease, liver disease and toxicology

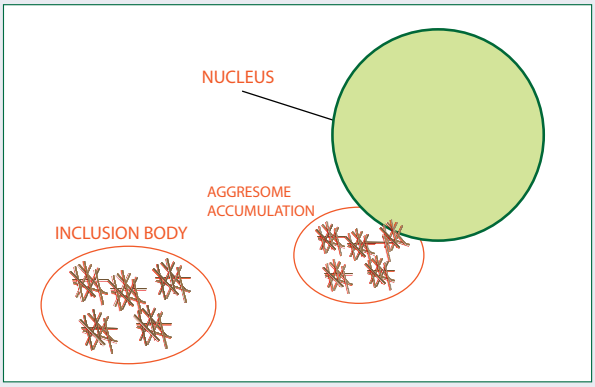
Pathological inclusion bodies containing aggregated proteins are characteristic of various neurodegenerative diseases, including Parkinson's, Alzheimer's and Huntington's disease. Aggresomes and related inclusion bodies may serve as storage depots for the disposal of toxic protein aggregates by the autophagy-lysosome pathway (ALP). One strategy for reducing cytotoxicity associated with aggregated protein accumulation is by enhancing their clearance through activation of autophagy.

PROTEOSTAT® Aggresome Detection Kit detects denatured protein cargo within autophagosomes in fixed cells and facilitates identification of small molecules that inhibit aggresome formation as well as enabling immuno-localization studies between aggregated protein cargo and the various proteins implicated in aggresome formation.

- Validated with small molecule modulators known to influence autophagy and proteasome pathways
- Provides a sensitive cell-based assay of drug responsiveness to identify inhibitors relevant to neurodegenerative disease in an authentic cellular context
- Fixed-cell assay is optimized for antibody co-localization studies to identify aggregated protein
- Easily quantifies aggresome and related inclusion bodies by flow cytometry
- Reliable and simple assay which doesn't require non-physiological protein mutations or genetically engineered cell lines

Mechanism of Action

Detection of aggregated protein cargo within aggresomes and inclusion bodies is based upon a molecular rotor dye. The non-fluorescent characteristic is due to the free rotation around a central carbon-carbon single bond, separating different aromatic portions of the probe. When the molecular rotor dye interacts with the cross-beta sheet quaternary structure of protein aggregates, it becomes highly fluorescent.



Aggresome Detection in Fixed Cells

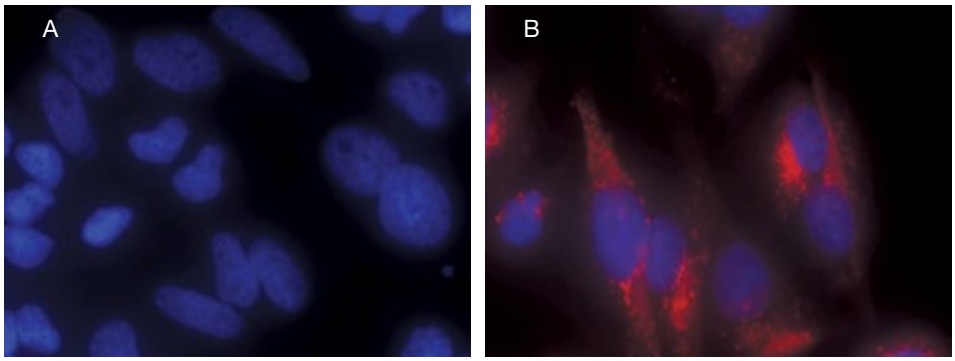


Figure 1: Aggresome detection in HeLa cells. Cells were treated for 12 hours (A) Control and (B) MG-132 (proteasome inhibitor). Post-treatment, cells were stained with PROTEOSTAT® Aggresome dye and Hoechst 33342.

AGGRESOME & INCLUSION BODY DETECTION

PROTEOSTAT® Dye Co-localized with Fluorescein-labeled LC3 Antibody Allows Specific Detection of Aggregated Proteins

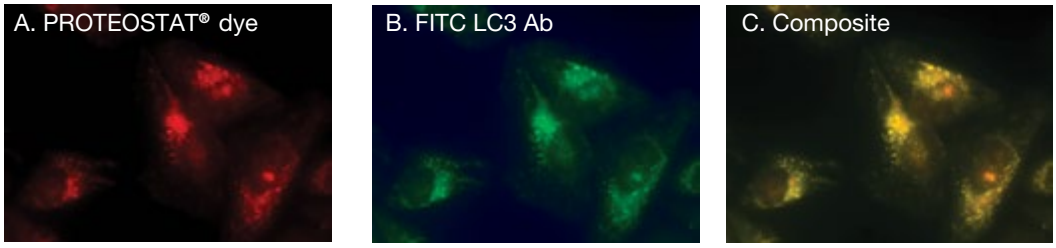


Figure 2: Cell Aggresome detected by PROTEOSTAT® dye co-localizes fluorescein-labelled antibody recognizing LC3I/II, as observed by fluorescence microscopy. HeLa cells were treated for 12 hours with 5 uM MG-132 on a slide and stained with (A) PROTEOSTAT® dye and (B) Fluorescein-labelled antibody recognizing LC3I/II; (C) composite image shown.

Identifying Protein Accumulation within Aggresomes by Flow Cytometry

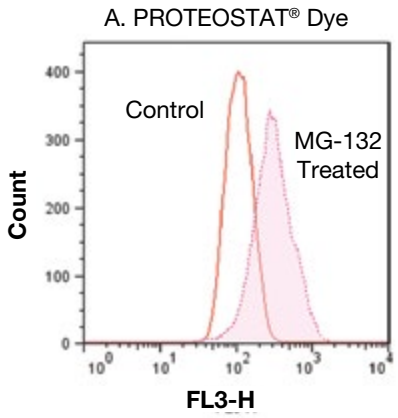


Figure 3A: Flow cytometry-based analysis. Jurkat cells were mock-induced with 0.2% DMSO or induced with 5 µM MG-132 overnight at 37°C. After treatment, cells were fixed and incubated with PROTEOSTAT® dye, then analyzed by flow cytometry without washing using a 488 nm laser in the FL3 channel. In MG-132 treated cells, fluorescent red signal increases about 3-fold. The described assay allows assessment of the effects of protein aggregation.

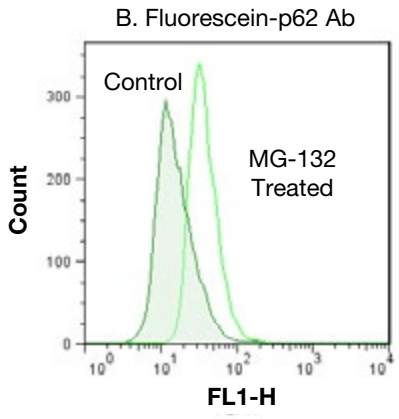


Figure 3B: Flow cytometry-based analysis. Jurkat cells were mock-induced with 0.2% DMSO or induced with 5 µM MG-132 overnight at 37°C. After treatment, cells were fixed and incubated with Fluorescein-p62 Ab (1:500 dilution of stock), then after washing, analyzed by flow cytometry using a 488 nm laser in the FL1 channel. In MG-132 treated cells, Fluorescein-p62 Ab signal increases about 2.5-fold.

Description	Cat. No.	Kit Components	
PROTEOSTAT® Aggresome Detection Kit	89409-190 / 10662-314	Dyes, MG-132, 10x assay buffer	
Dyes Included in Kit	**Excitation/Emission**	**Platform**	**Cell Type**
PROTEOSTAT® Aggresome Detection Reagent Hoechst 33342 Nuclear Stain	~ 500/600nm 350/461nm		Fixed

Citations

1. Raju I, Kumarasamy A, Abraham EC. Multiple Aggregates and Aggresomes of C-Terminal Truncated Human αA-Crystallins in Mammalian Cells and Protection by αB-Crystallin. PLoS One. 2011 May 12, 6 (5):e19876.
2. Shen, D., Coleman, Chan, E., Nicholson, T., Dai, L., Sheppard, P. and Patton, W. "Novel cell- and tissue-based assays for detecting misfolded and aggregated protein accumulation within aggresomes and inclusion bodies" (2011) Cell Biochemistry and Biophysics 60 (3) 173-185.

Application Note

1. Cell-Based Screening of Focused Bioactive Compound Libraries: Assessing Small Molecule Modulators of the Canonical Wnt Signaling and Autophagy-Lysosome Pathways.

APOPTOSIS/NECROSIS

GFP-CERTIFIED® APOPTOSIS/NECROSIS DETECTION KIT (89165-866 / 89165-868)

Multiplex assay that distinguishes between healthy, early apoptotic, late apoptotic and necrotic cells, compatible with GFP and other green fluorescent probes

Apoptosis is the best-characterized type of programmed cell death because of its importance in development and homeostasis, and in the pathogenesis of different diseases such as cancer. Apoptotic cells die in a controlled fashion in response to a variety of extrinsic or intrinsic signals (e.g., activation of TNF receptors, DNA damage, mitochondrial pathways). One of the hallmarks of apoptotic cell death include exposure of phosphatidylserine on the extracellular face of the plasma membrane.

Necrosis is an uncontrolled cell death characterized by cell swelling, as well as destruction of the plasma membrane and subcellular organelles, without nuclear fragmentation and condensation. Necrotic cell death is considered a heterogeneous phenomenon including both programmed and accidental cell death.

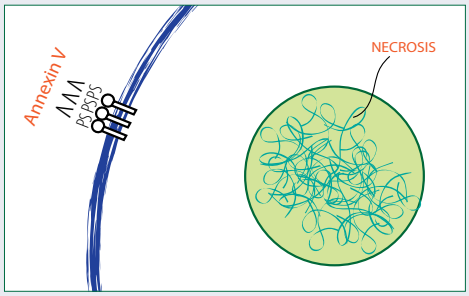
Phospholipid-binding proteins such as Annexin V bind with a high affinity to phosphatidylserine (PS) in the presence of Ca^{2+}. Given that Annexin V is not cell permeable, the binding of externalized PS is selective for early apoptotic cells. Similarly, loss of plasma membrane integrity, as demonstrated by the ability of a membrane-impermeable DNA intercalating dye to label the nucleus, represents a straightforward approach to demonstrate late stage apoptosis and necrosis.

GFP-CERTIFIED® Apoptosis/Necrosis Detection System was specifically designed for use with GFP-expressing cell lines and cells expressing blue or cyan fluorescent proteins (BFPs, CFPs). Additionally, the kit is suitable for use with live or post-fixed cells in conjunction with probes, such as labeled antibodies, or other fluorescent conjugates displaying similar spectral properties as fluorescein or coumarin.

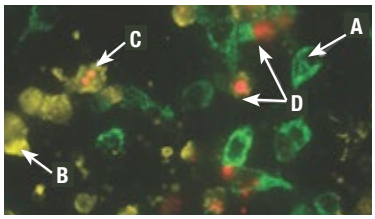
- True multiplexing capabilities with GFP and other green fluorescent probes
- Suitable for death pathway analysis and real-time drug/toxin studies
- Readily distinguishes between healthy, early apoptotic, late apoptotic and necrotic cells

Mechanism of Action

In the early stages of apoptosis, phosphatidylserine (PS) flips from the inside to the outside of the cellular membrane with the cellular membrane still intact. Enzo Gold-Annexin V has very high affinity to PS therefore immediately detects early apoptotic cells. Necrosis detection reagent is a membrane impermeable DNA intercalating dye that cannot penetrate a healthy cell membrane. In the late stages of apoptosis or during necrosis there is a loss of plasma membrane integrity, allowing the necrosis detection reagent to label the nucleus.



Analyzes More Stages Than Simple Annexin V-based Kits



A. Viable cells
B. Early apoptotic cells
C. Late apoptotic cells
D. Necrotic cells

Figure 1: GFP-CERTIFIED® Apoptosis/Necrosis Detection Kit (89165-866 / 89165-868) detects four distinct cell states. Mitochondrial GFP-expressing HeLa cells were treated with 2µM Staurosporine for 4 hours. The Apoptosis Detection Reagent (Gold) and Necrosis Detection Reagent (Red) specifically detect cell states with clear spectral separation from mitochondria-associated GFP signal. Healthy cells (A), cells undergoing apoptosis (B), cells undergoing late-stage apoptosis (C), and necrotic cells (D).

APOPTOSIS/NECROSIS

Real-time Detection of Apoptosis/Necrosis in Drug Screening

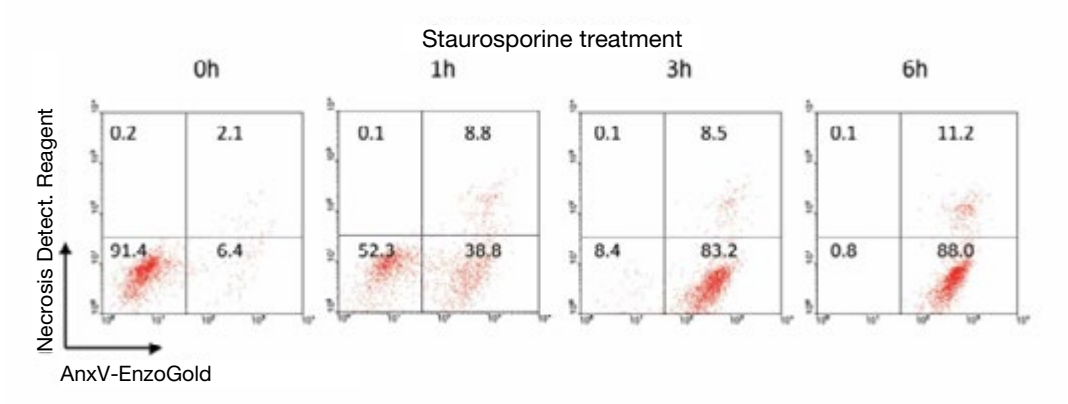


Figure 2: Jurkat cells stimulated with 2.5 µM Staurosporine for 0-6 h. Flow cytometry results using Jurkat suspension cells, showing early apoptotic (Annexin V-EnzoGold) and late apoptotic/necrosis (Annexin V-EnzoGold and necrosis stain).

True Multiplexing with GFP and Other Green Fluorescent Probes

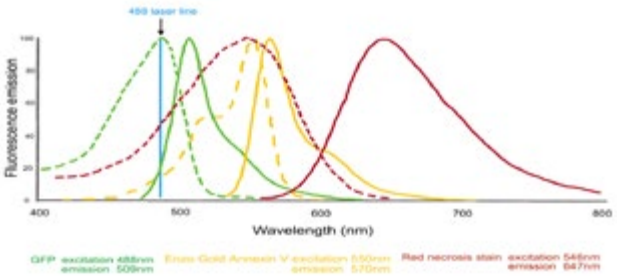


Figure 3: Excitation (dashed) and emission (solid) spectra for GFP, Annexin V-EnzoGold conjugate and Necrosis Detection Reagent (Red). All three fluorophores are readily excited with a 488nm laser source. The emission maxima of all fluorophores are well separated from one another.

Description	Cat. No.	Kit Components	
GFP-CERTIFIED® Apoptosis/Necrosis Detection Kit	89165-866 / 89165-868	Dyes, Staurosporine, 10x binding buffer	
Dyes Included in Kit	**Excitation/Emission**	**Platform**	**Cell Type**
Annexin V-EnzoGold Red necrosis stain	550nm/570nm ● 546nm/647nm ●		Live, Fix after incubation with dye and treatment

ANNEXIN V-FITC APOPTOSIS DETECTION KIT (89155-876 / 89155-878)

Easy, quick assay to distinguish between apoptotic cells and necrotic cells

- Quick 10-minute staining procedure
- Detection can be analyzed by flow cytometry or by fluorescence microscopy
- Differentiate between apoptosis and necrosis
- Includes propidium iodide to distinguish necrotic cells

Description	Cat. No.	Kit Components	
Annexin V-FITC Apoptosis Detection Kit	89155-876 89155-878	Dyes,4x binding buffer	
Dyes Included in Kit	**Excitation/Emission**	**Platform**	**Cell Type**
Annexin V-FITC Propidium Iodide	488/530nm ● 535/617nm ●		Live

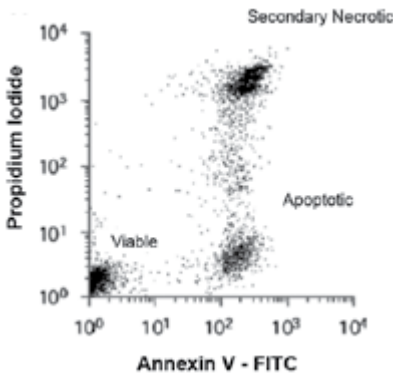


Figure 1: Easily distinguish between apoptotic and necrotic cells.

NUCLEAR-ID® GREEN CHROMATIN CONDENSATION KIT (89165-906)

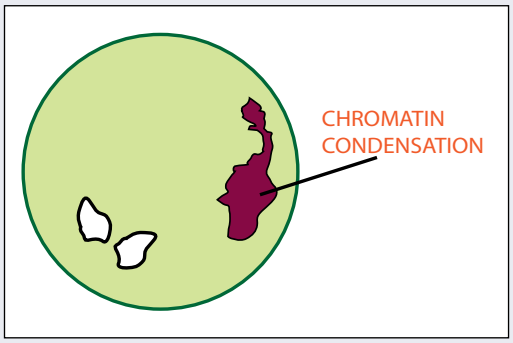
A highly permeable green-emitting dye for enhanced detection of apoptosis-induced chromatin condensation

Apoptosis is recognized as a pathway of highly orchestrated signaling events, and of critical importance in biological processes and pathologies including development, aging and cancer. Nuclear condensation is one of the more prominent hallmarks of the many morphological features associated with apoptosis, including cell membrane blebbing, cell shrinkage, nucleosomal fragmentation, and formation of fragmented apoptotic bodies.

- Intercalating dye with superior permeability with any live cell line
- Easy no-wash, mix and read protocol
- Eliminates need for specialized 350nm UV laser required for Hoechst dyes and reduces chances for channel interference
- No interference from small molecule fluorescence or cell autofluorescence

Mechanism of Action

The cell-permeable dye used in this application is an aromatic, planar cationic structure that inserts between stacked base pairs on the DNA duplex, providing an environmentally dependent fluorescence enhancement of the dye molecules and large increases in fluorescence signal relative to the free dye in solution. Since the signal enhancement provides a proportional response, direct quantitation of DNA is possible. Further signal increase is observed upon DNA condensation during apoptosis. Considering the general mutagenic effect of nucleic acid binding dyes, careful storage and handling of this dye is recommended.



Detect Apoptotic Chromatin Condensation

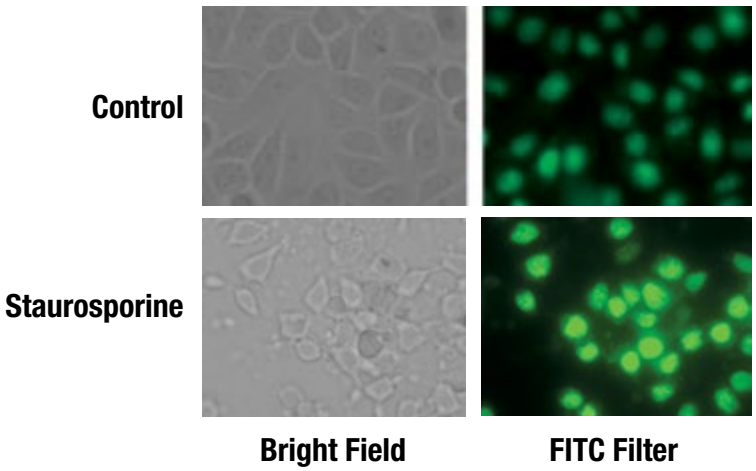
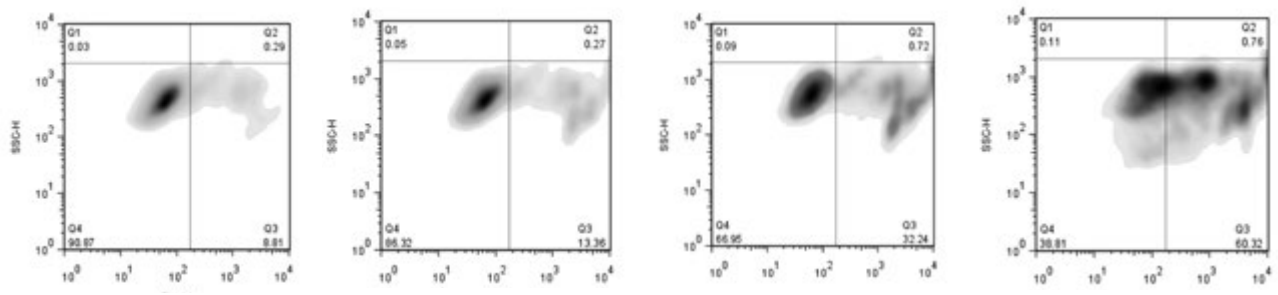


Figure 1: Chromatin condensation as observed by fluorescence microscopy using a standard 488nm laser. HeLa cells were treated for 4 hours with DMSO (Control) or 2 µM Staurosporine on a slide and stained with 5 µM NUCLEAR-ID® Green dye. The intercalating dye exhibits increased fluorescence upon chromatin condensation, a hallmark of apoptosis.

SENESCENCE

Flow Cytometry Compatible Assay for Toxicity Screening



Control **10 µM BPA** **10 µM Permatherin** **10 µM Rotenone**

Figure 2: Flow cytometry analysis of chromatin condensation in response to application of environmentally toxic compounds. Analysis was performed following 16-hour treatment of 1 x 10^6 cells/ml Jurkat cells with various compounds at the indicated concentration. The 488nm excitable green-emitting dye eliminates the need for specialized UV lasers required by Hoechst dye-based assays.

Description	Cat. No.	Kit Components	
NUCLEAR-ID® Green Chromatin Condensation Kit	89165-906	Dyes, Staurosporine, 10x assay buffer	
Dyes Included in Kit	**Excitation/Emission**	**Platform**	**Cell Type**
NUCLEAR-ID® Green Cell Cycle Detection Reagent	503/531nm ●		Live, Permeabilized Fixed

Citation

1. Okazawa S., Furusawa Y., Kariya A., Hassan M.A., Arai M., Hayashi R., Tabuchi Y., Kondo T., Tobe K. "Inactivation of DNA-dependent protein kinase promotes heat-induced apoptosis independently of heat-shock protein induction in human cancer cell lines." PLoS One. (2013) 8(3):e58325.

CELLULAR SENESCENCE LIVE CELL ANALYSIS ASSAY (10001-774)

Simple assay to quantify senescence-associated β-galactosidase activity in live cells

Cellular senescence, which is also known as biological aging, refers to permanent cell-cycle arrest, when cells are no longer capable of dividing but are metabolically active. Senescent cells are characterized by an irreversible G1 growth arrest involving the repression of genes that drive cell cycle progression and the upregulation of cell cycle inhibitors like p16INK4a, p53, and its transcriptional target, p21CIP1. They are resistant to mitogen induced proliferation, and assume a characteristic enlarged, flattened morphology. Research into the pathways that positively regulate senescence and ways cells bypass senescence is therefore critical in understanding carcinogenesis. Normal cells have several mechanisms in place to protect against uncontrolled proliferation and tumorigenesis.

Senescent cells show common biochemical markers such as expression of an acidic senescence-associated β-galactosidase (SA-β-gal) activity. While senescence has been characterized primarily in cultured cells, there is also evidence that it occurs in vivo. Cells expressing markers of senescence such as SA-β-gal have been identified in normal tissues.

- Quantify senescence-associated β-galactosidase activity in live cells using flow cytometry
- Sensitive assay kit uses fluorogenic substrate to measure SA-β-gal activity
- Simple protocol with no permeabilization required

Description	Cat. No.	Kit Components	
Cellular Senescence Live Cell Analysis Assay	10001-774	Dye, 100x cell pretreatment solution	
Dyes Included in Kit	**Excitation/Emission**	**Platform**	**Cell Type**
SA-β-gal Substrate	485/520nm ●		Live

VIABILITY

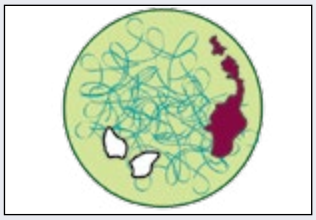
NUCLEAR-ID® CELL VIABILITY REAGENTS (89166-064 / 89166-066 / 89166-068)

Distinguish between live and dead cells in a single reagent

- Single easy-to-use reagent contains two nucleic dyes to distinguish between live and dead cells
- Cell permeable
- Available in a variety of combinations to enable multiplexing

Mechanism of Action

NUCLEAR-ID® Cell Viability Reagents contain a mixture of two nucleic acid binding dyes: 1) a cell-permeable nucleic acid dye that binds to all nuclei and can identify all cells and 2) a cell-impermeable nucleic acid dye that is suited for staining dead nuclei.



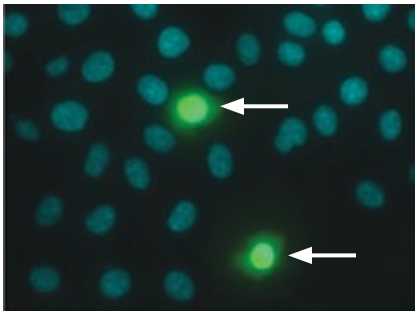


Figure 1: The NUCLEAR-ID® Blue/Green dye (89166-064) is detected as blue-stained nuclei in live cells and fluorescent-green nuclei in dead cells (arrows).

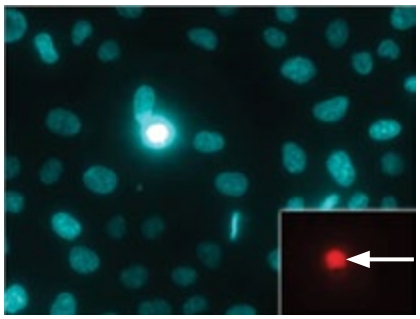


Figure 2: NUCLEAR-ID® Blue/Red dye (9166-066) is detected as blue-stained nuclei in live cells and fluorescent-red nuclei in dead cells (inset, arrow).

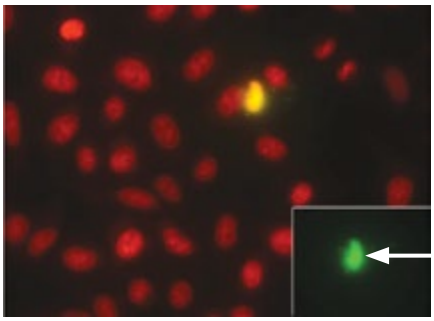


Figure 3: NUCLEAR-ID® Red/Green dye (89166-068) is detected as red-stained nuclei in live cells and fluorescent-green nuclei in dead cells (inset, arrow).

Description	Cat. No.	Excitation/Emission	Platform	Cell Type
NUCLEAR-ID® Blue/Green Cell Viability Reagent	89166-064	Live - 350/461nm ● Dead - 503/524nm ●		Live
NUCLEAR-ID® Blue/Red Cell Viability Reagent	89166-066	Live - 350/461nm ● Dead - 571, 619/639nm ●		Live
NUCLEAR-ID® Red/Green Cell Viability Reagent	89166-068	Live - 568/632nm ● Dead - 503/524nm ●		Live

Application Notes

1. Multiplexed Assay for IL-6 Secretion and Cell Viability Using an Epithelial Ovarian Cancer Cell Line
2. Validation of a Novel Tumoroid-Based Cell Culture Model to Perform 3D in vitro Cell Signaling Analyses

CELL FUNCTION

Enzo provides information-rich tools to quantify a range of functional responses in living cells

In addition to morphology, subcellular organelle functional response is a useful parameter for assessing cellular physiology. This includes phenomena such as nuclear DNA replication during cell cycle progression, receptor-triggered calcium mobilization, from the endoplasmic reticulum, reactive oxygen species generation in the mitochondria, and phospholipid redistribution into the plasma membranes of the daughter cells upon proliferation. These can all be modulated by various chemical, biological and environmental agents.

Cell cycle progression is controlled by a complex interplay among various cell cycle regulators that either stimulate or inhibit the cell from entering each stage of the process. These regulators activate transcription factors, which bind to DNA and turn on or off the production of proteins that result in cell division. Dysfunction of any step in this regulatory cascade causes abnormal cell proliferation which underlies many human pathological conditions, such as cancer. A crucial step to understanding these conditions is the ability to understand the mechanisms underlying alterations in the cell cycle.

The calcium ion is an important second messenger involved in many physiological and signal transduction processes within the cell. Calcium mobilization from intracellular stores in response to the activation of Gαq-coupled GPCRs is considered a standard approach to the pharmacological characterization of receptors and compounds, and is frequently implemented in primary screening, as well as to generate a compound structure activity relation (SAR) for lead development programs.

Oxidative stress is involved in regulating various growth, differentiation and death processes including apoptosis. A number of diseases are associated with excessive ROS generation, produced mostly in the mitochondria as byproducts of cell respiration or alternatively arising from activation of circulating neutrophils. Once produced within a cell, free radicals can damage a wide variety of cellular constituents, including proteins, lipids and DNA. However, at lower concentrations these very same agents may serve as second messengers in cellular signaling.

Incorporating lipophilic fluorescent probes into the plasma membrane is a convenient and simple approach to monitoring cell proliferation. Freshly labeled cells will have a particular fluorescence intensity value which will be reduced by one half upon each successive cell division, which can readily be quantified by flow cytometry.

Multidrug Resistance
EFLUXX-ID® Multidrug Resistance Assay Kits

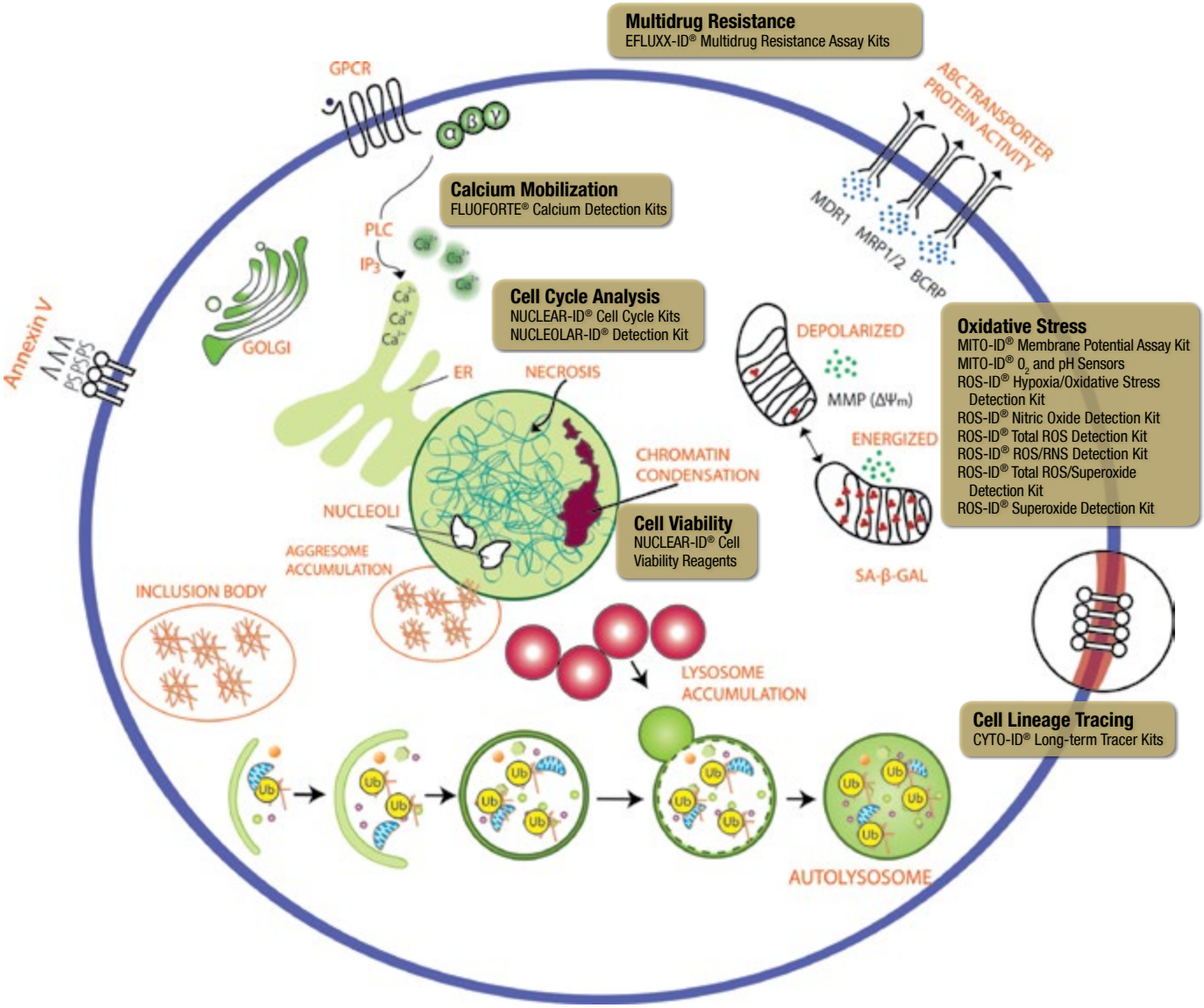
Calcium Mobilization
FLUOFORTE® Calcium Detection Kits

Cell Cycle Analysis
NUCLEAR-ID® Cell Cycle Kits
NUCLEOLAR-ID® Detection Kit

Cell Viability
NUCLEAR-ID® Cell Viability Reagents

Oxidative Stress
MITO-ID® Membrane Potential Assay Kit
MITO-ID® O_2 and pH Sensors
ROS-ID® Hypoxia/Oxidative Stress Detection Kit
ROS-ID® Nitric Oxide Detection Kit
ROS-ID® Total ROS Detection Kit
ROS-ID® ROS/RNS Detection Kit
ROS-ID® Total ROS/Superoxide Detection Kit
ROS-ID® Superoxide Detection Kit

Cell Lineage Tracing
CYTO-ID® Long-term Tracer Kits



CELL CYCLE ANALYSIS

NUCLEAR-ID® CELL CYCLE ANALYSIS KITS (89165-878 / 89165-890)

Cell cycle progression results independent of incubation time, temperature, dye and cell concentrations

Enzo Life Sciences NUCLEAR-ID® Cell Cycle Analysis Kits provide a convenient approach for studying the induction and inhibition of cell cycle progression by flow cytometry and microscopy. The kit is suitable for (1) determining the percentage of cells in a given sample that are in G_0/G_1, S and G_2/M phases, as well as to quantify cells in the sub-G_1 phase, and (2) DNA studies in live, permeabilized and fixed cells for normal cell lines and cell lines exhibiting multiple ploidy levels. A control cell cycle perturbation agent, nocodazole, is provided for monitoring changes in cell cycle dynamics. Potential applications for live-cell studies are in the determination of cellular DNA content and cell cycle distribution for the detection of variations in growth patterns, for monitoring apoptosis, and for evaluating tumor cell behavior and suppressor gene mechanisms.

- Cell cycle results independent of incubation time, temperature, dye and cell concentrations
- Monitors changes in cell cycle dynamics arising from drug treatment or other perturbations
- Performance validated using a wide range of cell densities
- No permeabilization step or RNase treatment necessary
- Simple “mix and read” protocol
- No photobleaching effect

Study Cell Cycle Progression

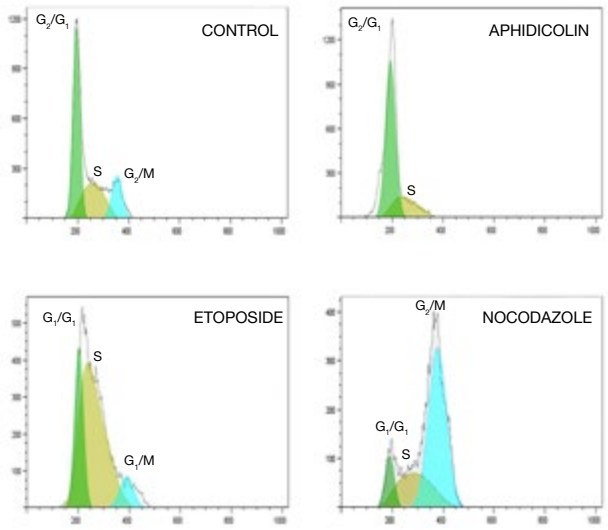


Figure 1: Drug treatments with live cells inhibit cell cycle progression at different phases.

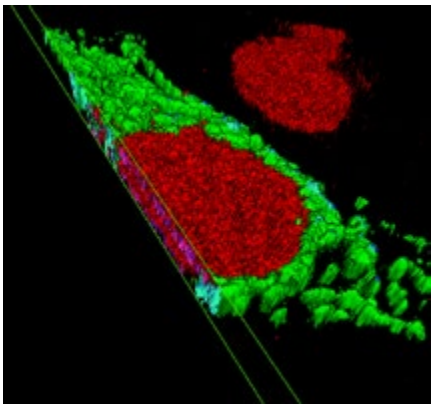


Figure 2: Three-dimensional reconstruction of the spatial relationship between the green fluorescent protein-expressing (GFP-expressing) mitochondria and the nucleus stained with NUCLEAR-ID® Red dye using a structured illumination method, implemented with the ApoTome from Carl Zeiss, Inc.

Description	Cat. No.	Kit Components	
NUCLEAR-ID® Cell Cycle Analysis Kits	89165-878 / 89165-890	Dye, Nocodazole, 10x assay buffer	
Dye Included in Kits	**Excitation/Emission**	**Platform**	**Cell Type**
89165-878 NUCLEAR-ID® Red Dye (GFP-CERTIFIED®)	568/637nm ●		Live, Permeabilized, Fixed
89165-890 NUCLEAR-ID® Green Dye	507/530nm ●		Live, Permeabilized, Fixed

CELL TRACKING & LINEAGE

CYTO-ID® LONG TERM CELL TRACER KITS (10136-012 / 10136-014)

Live cell fluorescent labeling over extended time periods with minimal toxic effect

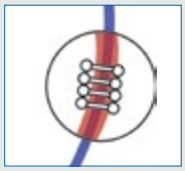
Long-term cell tracer dyes are well retained by cells for up to 96-hours after loading, and are passed to daughter cells upon mitosis. Since the dyes do not covalently modify proteins within the cells, normal physiological responses are better preserved than with molecular probes based upon thiol-reactive, chloromethyl-based or amine-reactive succinimidyl ester-based fluorescent dyes.

The CYTO-ID® Long-term Tracer Kits are suitable for tracing cell lineages, as well as assaying proliferation, precursor frequency, chemotaxis, migration, phagocytosis, and cell- and antibody-mediated cytotoxicity. Analysis of labeled and unlabeled cell populations over time by flow cytometry or microscopy is also feasible.

- Allows multiplex labeling with a variety of CELLESTIAL® fluorescent probes
- Minimal transfer of fluorescence from dye-labeled to unlabeled cells
- Suitable for long-term cell viability, cytotoxicity, cell adhesion, cell migration and cell-cell fusion assays
- Fluorescent labeling of live cells over an extended period of time, up to 96 hours
- Non-toxic to cells, as determined using the benchmark MTT cell viability assay

Mechanism of Action

These long-term cell tracer dyes use proprietary non-covalent cell labeling technology to stably incorporate a fluorescent dye containing hydrophobic aliphatic chains into the cell membrane's lipid bilayer. The dye is not toxic to cells so it is well retained by cells for up to 96 hours after loading, and is passed to daughter cells upon mitosis, and then they in-turn, exhibit half the fluorescence value.



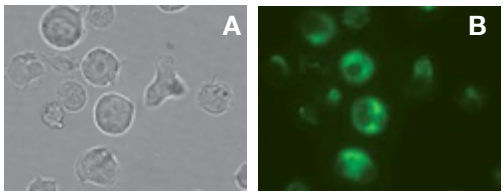


Figure 1: Composite bright-field (A) and fluorescence microscopy images (B) demonstrating staining of Jurkat cells with CYTO-ID® Green Tracer dye. Standard FITC (Green) filter set was used to image the membrane-bound signal.

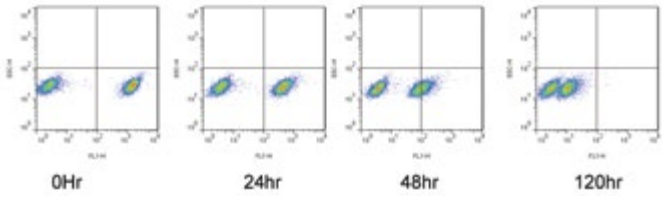


Figure 2: Flow cytometry analysis of fluorescence of mixed population of Jurkat cells over time. Jurkat cells stained with CYTO-ID® Green Tracer dye were mixed with an unstained population of Jurkat cells and incubated over a 120-hour period.

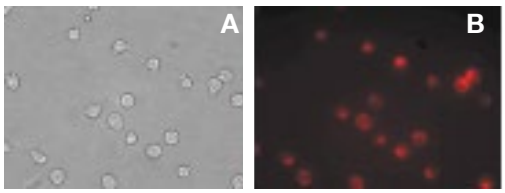


Figure 3: Composite bright-field (A) and fluorescence microscopy (B) images demonstrating staining of Jurkat cells with CYTO-ID® Red Tracer dye. Standard Texas Red filter set was used to image the membrane-bound signal.

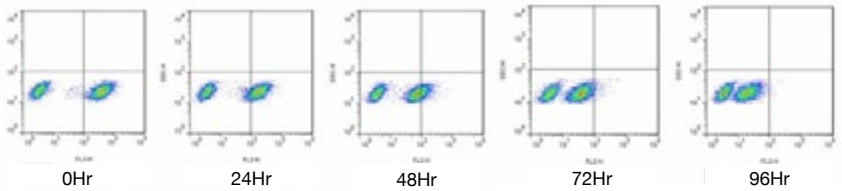


Figure 4: Flow cytometry analysis of fluorescence of mixed population of Jurkat cells over time. Jurkat cells stained with CYTO-ID® Red Tracer dye were mixed with an unstained population of Jurkat cells and incubated over a 96-hour period.

Description	Cat. No.	Kit Components	
CYTO-ID® Long Term Cell Tracer Kits	10136-012 / 10136-014	Dye, 4x labeling buffer, 10x HBSS	
Dyes Included in Kits	**Excitation/Emission**	**Platform**	**Cell Type**
10136-012 CYTO-ID® Green Tracer Dye	359, 460/527nm ●		Live
10136-014 CYTO-ID® Red Tracer Dye	450, 570/583nm ●		Live

CALCIUM MOBILIZATION

FLUOFORTE® CALCIUM MOBILIZATION KITS (89165-894 / 89165-896, 89165-902 / 89165-904)

A brighter, more robust fluorescent assay for calcium mobilization

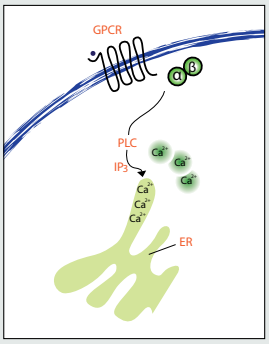
Measurement of transient calcium mobilization from intracellular stores in response to the activation of G protein-coupled receptors (GPCRs) is considered a standard approach to the pharmacological characterization of receptors and compounds, frequently implemented in primary screening and lead development programs.

FLUOFORTE® Calcium Assay Kits detect mobilization of intracellular calcium utilizing a fluorogenic calcium-binding dye optimized for superior cell-permeability and retention. The self-quenching dye undergoes an electronic change upon binding of calcium, resulting in a several order of magnitude greater fluorescence.

- Dye optimized for superior cell-permeability and retention
- Economical alternative developed for use with conventional dual-dispensing microplate readers, (i.e. BioTek, BMG Labtech, etc.)
- Provides EC50 values comparable to Fluo-4 and Calcium 4
- 2-fold brighter fluorescence vs. Fluo-4
- Sensitive dye provides larger assay window allowing for detection of even weak signal compound responses
- Better able to detect native levels of GPCR expression in cultured cells
- Data suitable for comparison with that obtained using alternative dyes

Mechanism of Action

The fluorogenic calcium-binding dye is provided to the cells as an acetoxymethyl (AM) ester, which is cell-permeable. Once inside the cells, the dye is hydrolyzed by intracellular esterases, which leads to generation of a cell membrane impermeable negatively charged form. In the absence of calcium, the calcium-binding moiety portion of the probe quenches the fluorescence of the fluorophore portion of the probe by photo-induced electron transfer. Binding of calcium relieves quenching and results in a several order of magnitude increase in the fluorescence emission intensity, with no shift in wavelength. The dye is capable of binding to physiologically relevant levels of calcium and increases in intracellular calcium lead to an increased fluorescence signal, which is readily measurable.



FLUOFORTE® AM Calcium Dyes are Brighter than Fluo-4 AM and Fluo-3 AM

FLUOFORTE® AM FLUO-4 AM FLUO-3 AM

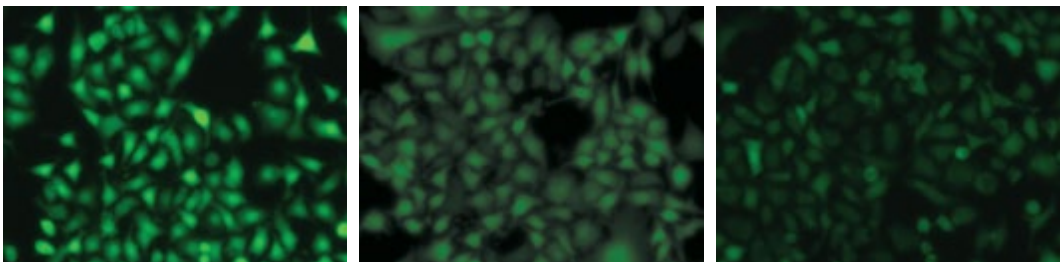


Fig. 1: U-2 OS cells were incubated with 100 µl of FLUOFORTE® AM, Fluo-4 AM, or Fluo-3 AM in HHBS at 37 °C for 1 hour. The cells were washed and ATP (20 µL/well) was added to achieve concentrations of 200 nM with dye efflux inhibitor. Cells were then immediately imaged in the FITC channel. FLUOFORTE® AM calcium dye exhibits significantly brighter fluorescence intensity than Fluo-4 AM and Fluo-3 AM.

CALCIUM MOBILIZATION

Obtain Robust Signal Intensity

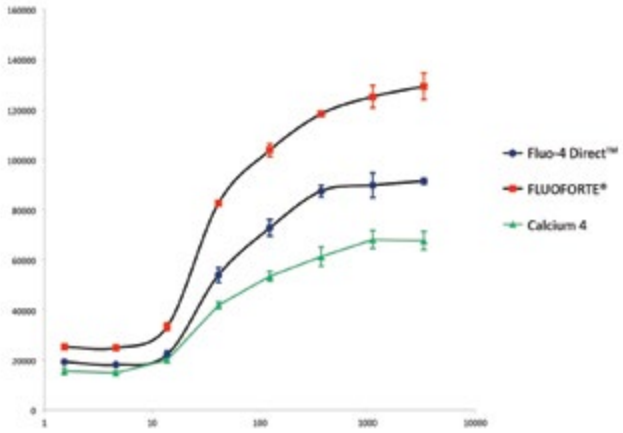


Figure 2: ATP dose response curves in CHO-M1 cells, expressing P2Y endogenous receptors. CHO cells were seeded overnight at 40,000 cells per 100 µl per well in a 96-well black wall/clear bottom microplate. The cells were incubated with 100 µl of Life Technologies' Fluo-4 Direct™ kit, Molecular Device's Calcium 4 kit (both based upon manufacturer's protocol) or FLUOFORTE® dye. ATP (20µl/well) was added by FlexStation to achieve the final indicated concentrations. Comparable ATP EC50 values were obtained using all three dyes, while FLUOFORTE® generated the highest intensity signal.

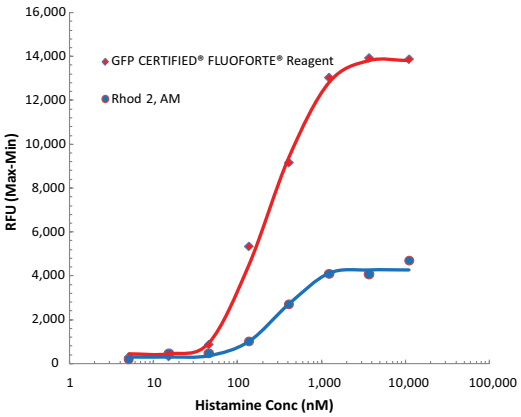
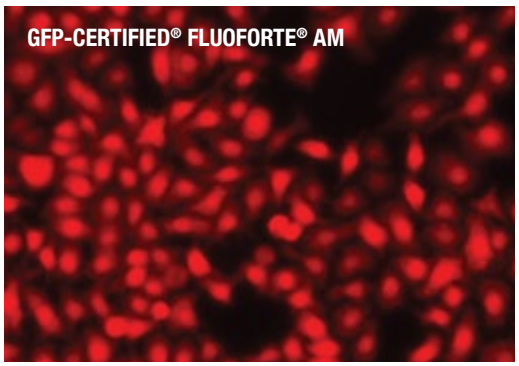


Figure 3: Histamine H1 receptors: HeLa cells were seeded overnight at 40,000 cells per 100 µL per well in a 96-well black wall/clear bottom microplate. The cells were incubated with 100 µL of GFP-CERTIFIED® FLUOFORTE® reagent or Rhod-2 AM dye for 1 hour at 37°C. Histamine (20 µL/well) was added using a BioTek two-syringe pump dispenser to achieve the final indicated concentrations. No significant difference in EC50 of Histamine for GFP-CERTIFIED® FLUOFORTE® and Rhod2-AM dye was observed. GFP-CERTIFIED® FLUOFORTE® reagent generated higher intensity signal and a larger assay window.

GFP-CERTIFIED® FLUOFORTE® is Brighter than Rhod-2 AM



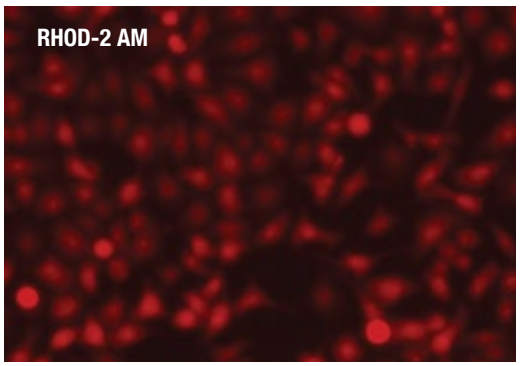


Figure 4: U-2 OS cells were incubated with GFP-CERTIFIED® FLUOFORTE® AM dye or Rhod-2 AM dye. Cells were washed and treated with a final concentration of 100nM ATP and imaged immediately. GFP-CERTIFIED® FLUOFORTE® AM dye exhibits brighter fluorescence intensity than Rhod-2 AM.

Description	Cat. No.	Kit Components	
FLUOFORTE® Calcium Mobilization Kits	89165-894 / 89165-896, 89165-902 / 89165-904	Dye, dye efluxx inhibitor	
Dye Included in Kits	**Excitation/Emission**	**Platform**	**Cell Type**
89165-894 / 89165-896 FLUOFORTE® Calcium Dye *	503/531nm ●		Live
89165-902 / 89165-904 GFP-CERTIFIED® FLUOFORTE® Calcium Dye **	530/570nm ●		Live

* FLUOFORTE® Reagent available separately as 89165-958, 89165-960
** GFP-CERTIFIED® FLUOFORTE® Reagent available separately as 89165-962 / 89165-964

OXIDATIVE STRESS

MITO-ID® MEMBRANE POTENTIAL KITS (10662-274 / 89165-898, 89165-900)

Monitors mitochondrial membrane energetic status using a simple mix-and-read, no-wash protocol

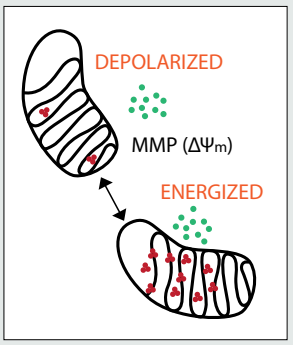
Mitochondria play a central role in cellular metabolism, bioenergetics, and apoptosis. Decreased mitochondrial function is known to be a major contributor to drug-associated toxicity in various organs. Growing FDA emphasis on evaluating the mitotoxic effects of drug candidates has increased the importance of determining such effects early in the drug development process.

The MITO-ID® Membrane Potential Cytotoxicity Kit measures fluctuations in mitochondrial membrane potential (MMP) utilizing a cationic dual-emission dye that exists as green fluorescent monomers in the cytosol, and accumulates as orange fluorescent J-aggregates in the mitochondria. Mitochondria having a low membrane potential will accumulate low concentrations of dye and will exhibit green fluorescence while more highly polarized mitochondria will exhibit orange-red fluorescence. Cells exhibit a shift from orange to green fluorescence as mitochondrial function becomes increasingly compromised. Unique HTS assay monitors mitochondrial membrane potential in real-time without wash step or medium removal.

- 10X more sensitive than JC-1 with superior aqueous solubility
- Photostable dual-emission dye
- No-wash/No-medium removal
- Separate MITO-ID® assay is available for detection of mitochondrial mass
- Suitable for high-throughput applications
- Detects toxicity at lower drug/dose concentrations
- No solvent artifacts as those seen with JC-1 formulation
- Suitable for time-course studies evaluating intact and compromised mitochondria

Mechanism of Action

The basic chemical structure of the dye consists of highly conjugated moieties that extensively delocalize a positive charge thus allowing electrophoretic uptake toward the negatively charged matrix phase of the polarized inner mitochondrial membrane. The dye is capable of entering selectively into mitochondria wherein it changes its color reversibly from green to orange as membrane potential increases (dual-emission potential probe). This photophysical property is due to the reversible formation of J-aggregates upon membrane polarization that causes shifts in emitted light from ~530nm (the emission of the monomeric dye) to 590nm (the emission of the J-aggregate form) when excited at 490nm. As a consequence, mitochondria having a low membrane potential will accumulate low concentrations of dye and will exhibit green fluorescence while more highly polarized mitochondria will exhibit orange-red fluorescence.



Dual-emission Probe Monitors Energetic Status

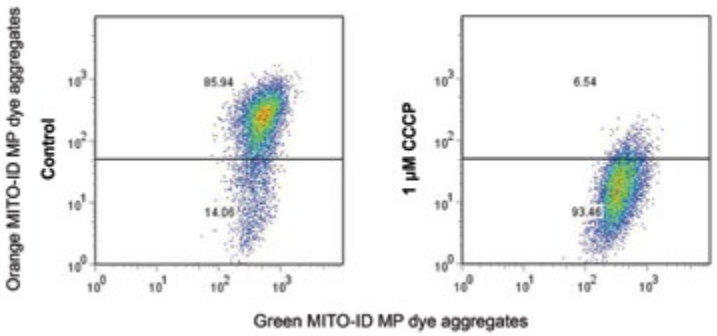


Figure 1: Flow Cytometric Analysis of Control and Treated Cells. Jurkat cells were untreated (left) and were treated with 1 µM CCCP for 15 mins (right). Cells were then stained with Enzo's MITO-ID® Membrane Potential dye (10662-274 / 89165-898), and run on a FACS Calibur instrument.

OXIDATIVE STRESS

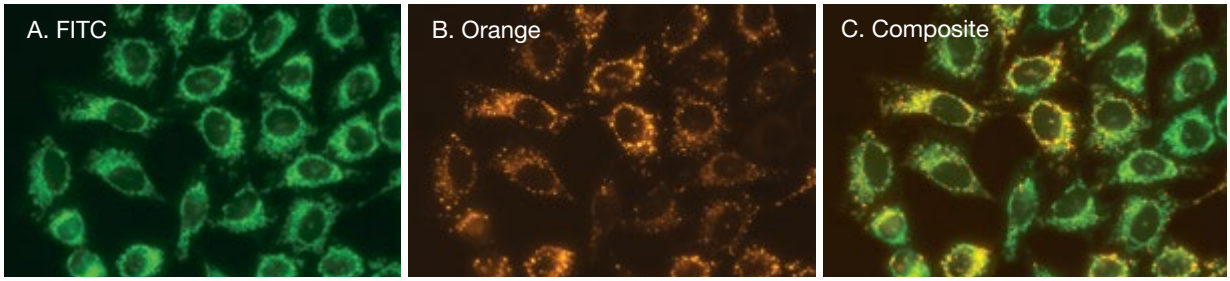


Figure 2: The mitochondria of HeLa cells were stained with MITO-ID® Membrane Potential dye and visualized by epifluorescence microscope. Orange fluorescent aggregates are localized in the mitochondria, while green fluorescent monomers mainly stain the cytosol.

Detect Mitochondrial Perturbations with 10 times More Sensitivity than JC-1

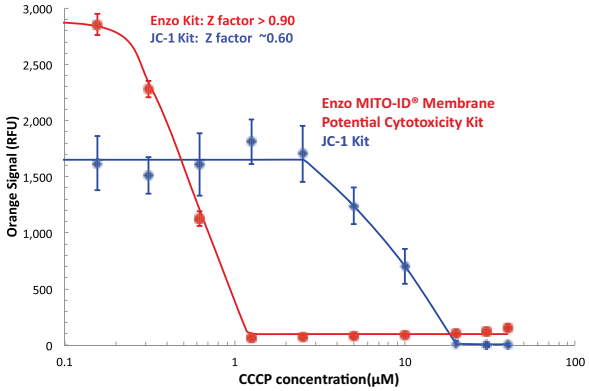


Figure 3: Mitochondrial membrane potential (MMP) was evaluated in HeLa cells treated with CCCP using MITO-ID® dye (red) or JC-1 (blue). Using a conventional fluorescence microplate reader, MMP was shown to decrease with increasing CCCP concentration as indicated by a decrease in orange fluorescence. Improved aqueous solubility of the dye and no-wash protocol minimizes variability, leading to a higher Z-factor (> 0.9) than that obtained with JC-1.

Real-time Detection of Mitotoxicity in Drug Screening

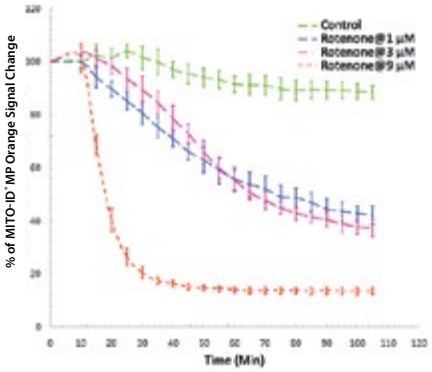


Figure 4: Time-course study of mitochondrial membrane potential changes using a BioTek Synergy™ Mx fluorescence microplate reader. HeLa cells were incubated with MITO-ID® MP dye for 30 minutes at room temperature (no serum or media removal). Rotenone was added to achieve concentrations of 1 µM, 3 µM and 9 µM. MITO-ID® MMP dye was shown to be responsive to rotenone, as demonstrated by a decrease in orange signal.

Description	Cat. No.	Kit Components	
MITO-ID® Membrane Potential Kits	10662-274 / 89165-898 89165-900	Dyes, CCCP, 10x assay buffer 1, 50x assay buffer 2	
Dyes Included in Kits	**Excitation/Emission**	**Platform**	**Cell Type**
10662-274 / 89165-898 MITO-ID® MP Detection Reagent Necrosis Detection Reagent	490/525 ●, 590nm ● 546nm/647nm ●		Live
89165-900 MITO-ID® MP Detection Reagent	490/525 ●, 590nm ●		Live

OXIDATIVE STRESS

MITO-ID® EXTRACELLULAR O_2 SENSOR KITS (10136-018 / 10136-032, 10136-034 / 10136-036)

High-throughput cell-based assay used to detect mitochondria dysfunction through real-time oxygen consumption measurements in whole cells

Oxygen consumption is one of the most informative and direct measures of mitochondrial function. Mitochondria have been shown to play a central role in cellular metabolism, bioenergetics, and apoptosis. Mitochondrial dysfunction is implicated in numerous disease states, including cancer, obesity, neurodegeneration and ischemia. A major mechanism of drug-induced toxicity has been linked to mitochondria dysfunction.

The MITO-ID® O_2 Extracellular Sensor Kit resolves limitations with the traditional methods of measuring oxygen consumption, includes low throughput and high complexity by providing a direct, real-time measurement of extracellular oxygen consumption rate (OCR) on a fluorescence plate reader.

- Phosphorescent probe increase in signal intensity with O_2 consumption (↓ O_2 levels)
- For use on standard fluorescence plate readers (96 or 384 well format), for high-throughput analysis
- Direct real-time kinetic analysis of extracellular oxygen consumption rates (OCR) as a measure of cellular respiration and mitochondrial function
- For use with whole cells (adherent and suspension), isolated mitochondria, permeabilized cells, 3D cultures, scaffolds and matrixes, tissues, small organisms, isolated enzymes, bacteria, yeasts and molds
- Multiplexing with MITO-ID® Extracellular pH Sensor Probe (10136-042) allows the simultaneous real-time measurement of mitochondrial respiration and glycolysis, and the analysis of the metabolic phenotype of cells, and the shift (flux) between the two pathways under pathological states
- Combine with MITO-ID® Membrane Potential Cytotoxicity Kit (89165-900) to confirm mitochondrial specific toxicity

Mechanism of Action

MITO-ID® Extracellular O_2 Sensor Kit (High Sensitivity), contains a phosphorescent oxygen sensitive reagent enabling high-throughput and real-time oxygen consumption readout. In this assay, MITO-ID® Extracellular O_2 Sensor Probe is quenched by oxygen, through molecular collision, and thus the amount of fluorescence signal is inversely proportional to the amount of extracellular oxygen in the sample. Rates of oxygen consumption are calculated from the changes in fluorescence signal over time. The reaction is non-destructive and fully reversible (neither MITO-ID® Extracellular O_2 Sensor Probe nor oxygen are consumed), facilitating measurement of time courses and drug treatments. The addition of high-sensitivity mineral oil (supplied) is used to limit back diffusion of ambient oxygen. The flexible plate reader format, allows multiparametric or multiplex combination with a range of other reagents.

Detect Mitochondrial Dysfunction Within Minutes of Treatment

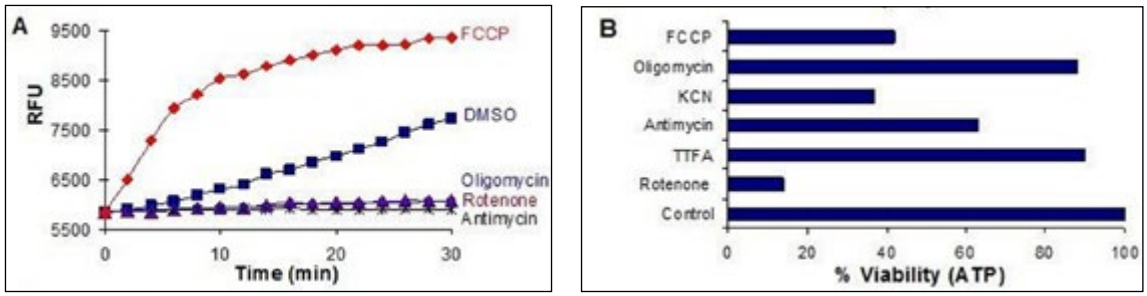


Figure 1. Assessment of mitochondrial function with MITO-ID® Extracellular O_2 Sensor Kit (HS) (A) or traditional ATP assay (B) following treatment with mitochondrial inhibitors (Oligomycin, Rotenone, Antimycin) and uncoupling agent (FCCP). Results illustrate that drug-induced mitochondrial dysfunction is evident immediately post-treatment (A) despite varying levels of viability at 24 hours by ATP assay (B).

OXIDATIVE STRESS

Description	Cat. No.	Kit Components	
MITO-ID® Extracellular O_2 Sensor Kits	76022-464 / 10136-032 10136-034 / 10136-036	Dye, MITO-ID® oil (ENZ-51044) / MITO-ID® High Sensitivity oil (ENZ-51045)	
Dye Included in Kits	**Excitation/Emission**	**Platform**	**Cell Type**
MITO-ID® Extracellular O_2 Sensor Probe	380/650nm ●		Live

Citations

1. S. Lisanti, et al. "Deletion of the Mitochondrial Chaperone TRAP-1 Uncovers Global Reprogramming of Metabolic Networks." Cell Rep. (2014) 8(3):671-677.

Application Notes

1. Hepatotoxicity: Measuring drug-induced mitochondrial toxicity in HepaRG™ cells using the MITO-ID® Extracellular O_2 Sensor Kit
2. Cardiotoxicity: Assessing Mitochondrial Toxicity in Stem Cell-Derived Cor.4U® Cardiomyocytes
3. Cellular Energy Flux in Real Time: Optimization of a multi-mode detection model for measuring real-time cellular respiration and mitochondrial function using fluorophoric biosensors

MITO-ID® INTRACELLULAR O_2 SENSOR PROBE (10136-038)

Sensitive probe for the measurement of intracellular oxygen concentration levels

- An easy mix and measure, 96 or 384 well fluorescence plate reader based approach for the analysis of intracellular oxygen concentration at the cell monolayer
- Time-resolved fluorescence (TR-F) measurements provide real-time assessment of oxygen levels in resting cells and their response to stimulation
- For measurement of intracellular oxygen concentration in whole cells (adherent), 3D cultures, scaffolds, tissues and small organisms
- Applications include hypoxia, 3D culture models and mitochondrial toxicity. Use with ROS-ID® Hypoxia/Oxidative Stress Detection Kit (10662-268 / 10136-016)
- Multiplex with MITO-ID® Extracellular pH Sensor Probe (10136-042) to confirm mitochondrial specific toxicity (icO_2 & ECAR)
- Use in parallel the MITO-ID® Extracellular Sensor Kit (High Sensitivity) (10136-034 / 10136-036) to obtain complete oxygen analysis in cells

Mechanism of Action

The MITO-ID® Intracellular O_2 Probe is a metalloporphyrin based oxygen-sensitive probe developed for the intracellular analysis of molecular oxygen using plate-based, time-resolved fluorometry. The assay is based on the ability of oxygen to quench the excited state of the oxygen-sensitive reagent. The probe is taken up via non-specific energy dependent endocytosis and, after washing, the cells are monitored on a TR-F plate reader. Probe phosphorescence is quenched by intracellular oxygen in a non-chemical, reversible manner allowing the measurement of average intracellular oxygen levels and facilitating real-time monitoring of relative changes in cellular oxygen concentration. The signal increases with a reduction in intracellular oxygen and decreases with an increase in intracellular oxygen. Probe signal can be converted to absolute oxygen concentrations following calibration of probe.

Description	Cat. No.	Excitation/Emission	Platform	Cell Type
MITO-ID® Intracellular O_2 Sensor Probe	10136-038	370/660nm ●		Live

OXIDATIVE STRESS

MITO-ID® EXTRACELLULAR PH SENSOR PROBE (10136-042)

A pH sensitive phosphorescent probe that can monitor cellular lactic acid extrusion, resulting from glycolysis, as a measure of glycolytic flux (ECA/ECAR)

Metabolic changes can play a critical role in a variety of disease states and toxicities. Insight into the two main cellular ATP generating pathways: glycolysis and oxidative phosphorylation, therefore is particularly informative when examining metabolic perturbations.

MITO-ID® Extracellular pH Sensor Probe is used for the assessment of extracellular acidification. As lactate production is the main contributor to this acidification, Enzo's pH Sensor Probe is a convenient and informative measure of cellular glycolytic flux. Such analysis of glycolytic activity helps with assessing alterations in glucose metabolism, detecting glycolytic inhibition and be used to detect mitochondrial dysfunction.

- For use on standard fluorescence plate readers (96 or 384 well format), for high-throughput analysis
- Real-time kinetic analysis of extracellular acidification rates (ECA/ECAR)
- Measures alterations in glucose metabolism and glycolytic function, such as is seen in Warburg
- For use with whole cells (adherent and suspension), permeabilized cells, 3D cultures, scaffolds and tissues
- Multiplexing with MITO-ID® Extracellular O_2 Sensor Kit (High Sensitivity) (10136-034 / 10136-036) allows the simultaneous real time measurement of mitochondrial respiration and glycolysis, and the analysis of the metabolic phenotype of cells, and the shift (flux) between the two pathways under pathological states

Efficiently Monitor Glycolytic Activity

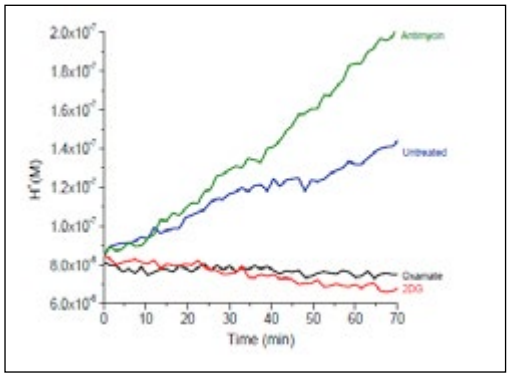


Figure 1. Acidification profiles of HepG2 cells treated with glucose transport inhibitors (2DG, Oxamate) or mitochondrial inhibitor (Antimycin) relative to control. Results show a decrease in acidification when cells are treated with glycolysis inhibitors.

Multiplex MITO-ID® O_2 and pH Sensors to Measure Cellular Energy Flux

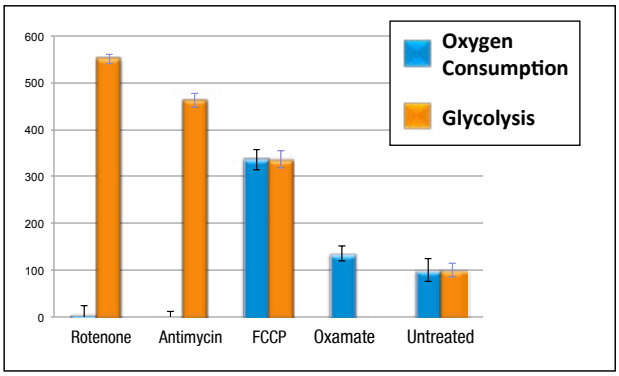


Figure 2. MITO-ID® Extracellular pH Sensor Probe paired with the MITO-ID® Extracellular O_2 Sensor Kit (10136-034 / 10136-036) allows the simultaneous real-time measurement of mitochondrial respiration and glycolysis and analysis of the metabolic phenotype of cells and flux between the two pathways under pathological states.

Description	Cat. No.	Excitation/Emission	Platform	Cell Type
MITO-ID® Extracellular pH Sensor Probe	10136-042	370/615nm ●		Live

Application Notes

1. Measuring Glycolysis as Extracellular Acidification
2. Cardiotoxicity: Assessing Mitochondrial Toxicity in Stem Cell-Derived Cor.4U® Cardiomyocytes
3. Cellular Energy Flux in Real Time: Optimization of a multi-mode detection model for measuring real-time cellular respiration and mitochondrial function using fluorophoric biosensors

OXIDATIVE STRESS

ROS-ID® TOTAL ROS/SUPEROXIDE DETECTION KIT AND RELATED KITS (89165-882 / 89165-864 / 89165-884 / 89165-886 / 89165-888)

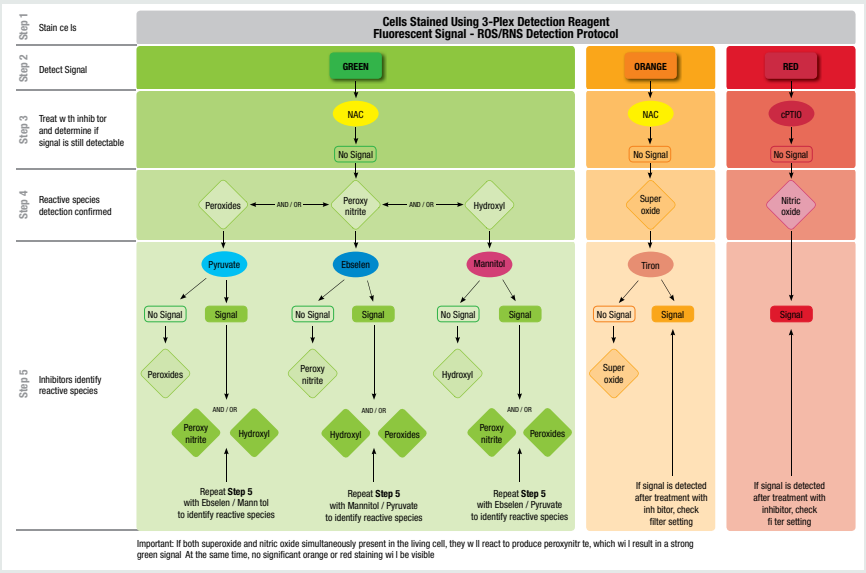
Free radicals and other reactive species play influential roles in many human physiological and patho-physiological processes, including cell signaling, aging, cancer, atherosclerosis, macular degeneration, sepsis, various neurodegenerative diseases (Alzheimer's and Parkinson's disease) and diabetes. Once produced within a cell, free radicals can damage a wide variety of cellular constituents, including proteins, lipids and DNA. However, at lower concentrations these very same agents may serve as second messengers in cellular signaling.

The ROS-ID® Total ROS/Superoxide Detection Kit enables detection of comparative levels of total ROS and also allows determination of superoxide production in live cells. Through the combination of two specific fluorescent probes, the kit provides a simple and specific assay for the real-time measurement of global levels of reactive oxygen species/reactive nitrogen species (ROS/RNS) and superoxide (SO) in living cells.

- Directly monitors reactive oxygen and reactive nitrogen species (ROS/RNS) in live cells
- Compatible with major components of tissue culture media (phenol red, FBS and BSA)
- High sensitivity, specificity and accuracy for live cell studies
- Simultaneously detects ROS and Superoxide
- With specific inhibitors, distinguish between different reactive species, such as hydrogen peroxide, peroxynitrite and hydroxyl radicals

Mechanism of Action

The strategy for fluorescence-based detection of NO employs an o-phenylenediamine scaffold, which in the presence of NO and air oxidizes to the corresponding aryl triazole, contributing its success of high selectivity for NO under aerated conditions. The oxidation of the ROS and Superoxide detection reagents produces green and orange fluorescent compounds, respectively. The Green detection reagent reacts with a broad range of reactive oxygen species and the Orange detection reagent reacts with superoxide (free radicals) but not other reactive oxygen species.



Profiling of ROS/Superoxide by Fluorescence Microplate Reader

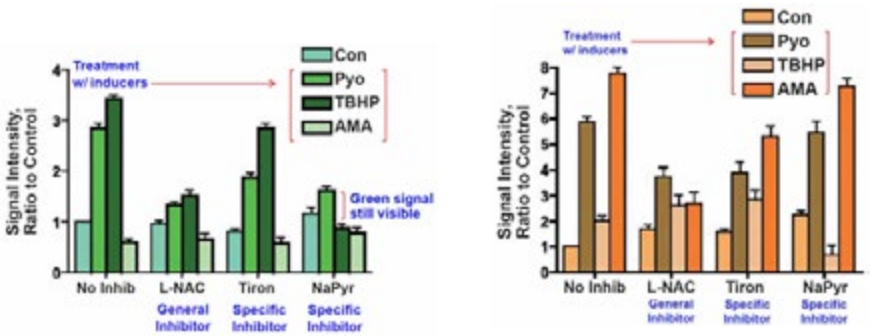


Figure 1: ROS-ID® Total ROS/Superoxide Detection Kit and a set of ROS scavengers/inhibitors were used to profile ROS production in HeLa cells treated with antimycin A, (AMA, specific superoxide inducer), t-butyl-peroxide (TBHP, specific peroxide inducer), and pyocyanin (general ROS inducer). Similar results were obtained using U-2 OS and CHO K1 cells.

OXIDATIVE STRESS

Profile Specific Reactive Oxygen/Nitrogen Species with Inhibitors

(A) HeLa Cells Loaded with ROS/RNS 3-Plex Detection Reagent for 2 h, 37° C and Induced for 20 min, 37° C

(B) Pretreatment with 5 mM NAC

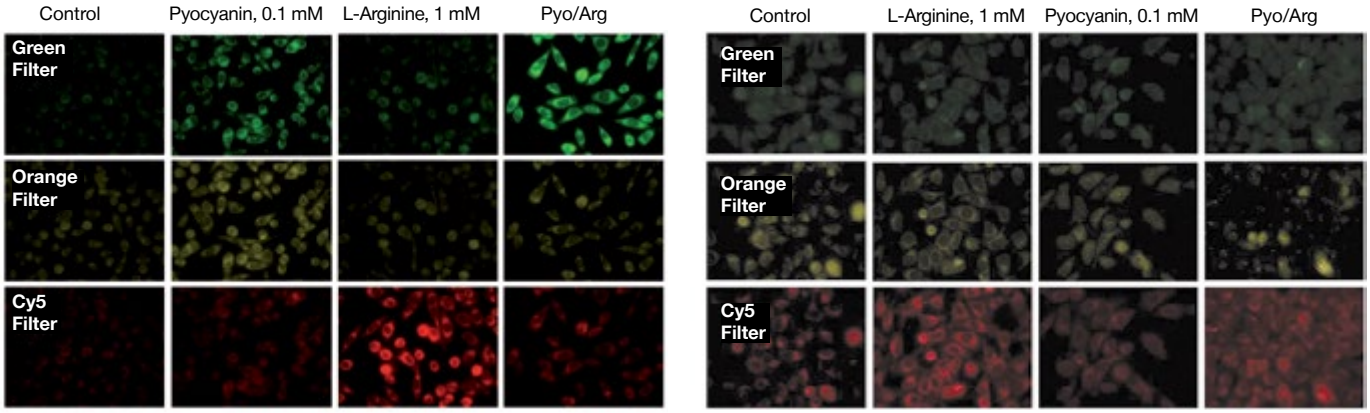


Figure 2: Specific profiling of reactive oxygen/nitrogen species formation by fluorescence microscopy in HeLa cells loaded with dyes from ROS-ID® ROS/RNS Detection Kit (89165-864). (A) Combined treatment of Pyocyanin and L-Arg generates peroxynitrite (green fluorescence) due to the reaction of NO with superoxide, but little NO (red fluorescence). (B) Pretreatment with NAC inhibits peroxynitrite and superoxide formation, but not NO.

Description	Cat. No.	Kit Components	Dyes Included	Ex/Em	Platform	Cell Type
ROS-ID® ROS/RNS Detection Kit	89165-864	Dyes, L-Arginine, Pyocyanin, C-PTIO, NAC, 10x wash buffer	Oxidative Stress Detection Reagent Superoxide Detection Reagent NO Detection Reagent	504/524nm ● 530/590nm ● 648/666nm ●		Live
ROS-ID® Total ROS/ Superoxide Detection Kit	89165-882	Dyes, Pyocyanin, NAC, wash buffer salts	Oxidative Stress Detection Reagent Superoxide Detection Reagent	504/524nm ● 530/590nm ●		Live
ROS-ID® Total ROS Detection Kit	89165-884	Dye, Pyocyanin, NAC, wash buffer salts	Oxidative Stress Detection Reagent	504/524nm ●		Live
ROS-ID® Superoxide Detection Kit	89165-886	Dye, Pyocyanin, NAC, wash buffer salts	Superoxide Detection Reagent	530/590nm ●		Live
ROS-ID® NO Detection Kit	89165-888	Dye, L-Arginine, C-PTIO, 10x wash buffer	NO Detection Reagent	648/666nm ●		Live

Citations

1. Huo X, et al. Deoxycholic Acid Causes DNA Damage while Inducing Apoptotic Resistance through NF-{kappa}B Activation in Benign Barrett's Epithelial Cells. Am J Physiol Gastrointest Liver Physiol. 2011 Aug;301(2):G278-86. Epub 2011 Jun 2.
2. Sun X, Sun GB, Wang M, Xiao J, Sun XB. Protective effects of cynaroside against H(2) O(2) -induced apoptosis in H9c2 cardiomyoblasts. J Cell Biochem. 2011 Aug;112(8) 2019-29.
3. Hayashi, K., Nakamura, M., Sakamoto, W., Yogo,T., Kori, T. and Ishimura, K. Formation of TiO2 Nanostructures by Enzyme-Mediated Self-Assembly for the Destruction of Macrophages Chem. Mater., 2011, 23 (14), 3341–3347.
4. Hoshikawa H, Indo K, Mori T, Mori N. Enhancement of the radiation effects by D-allose in head and neck cancer cells. Cancer Lett. 2011 Jul 1;306(1):60-6.
5. Yang L, Wu D, Wang X, Cederbaum AI. Depletion of cytosolic or mitochondrial thioredoxin increases CYP2E1-induced oxidative stress via an ASK-1-JNK1 pathway in HepG2 cells.Free Radic Biol Med. 2011 Jul 1;51(1):185-96.
6. Wu D, Wang X, Zhou R, Cederbaum A. CYP2E1 enhances ethanol-induced lipid accumulation but impairs autophagy in HepG2 E47 cells. Biochem Biophys Res Commun. 2010 Nov 5;402(1):116-22

Application Note

1. Screening Reactive Oxygen Species (ROS) on IQUE® Screener

HYPOXIA

ROS-ID® HYPOXIA/OXIDATIVE STRESS DETECTION KIT (10662-268 / 10136-016)

Specific detection system for simultaneous analysis of hypoxia and oxidative stress

The Warburg effect describes the metabolic shift from oxidative phosphorylation to aerobic glycolysis that takes place in tumors, perhaps, even before the inevitable hypoxia that occurs as the tumor outgrows its insufficient and often irregular vasculature. The culmination of metabolic changes in cancer cells contributes significantly to increased metastasis and drug-resistance, aspects known to increase patient mortality. Based on these observations, proteins that mediate metabolism and directly target hypoxic cells in primary, metastatic tumors are attractive targets for therapeutic intervention.

The ROS-ID® Hypoxia/Oxidative Stress Detection Kit is designed for functional detection of hypoxia and oxidative stress levels in live cells. This kit includes fluorogenic probes for hypoxia (red) and for oxidative stress levels (green) as two major components.

- Single-step detection of hypoxia and oxidative stress with high sensitivity, selectivity and accuracy in live cells
- Complete set of reagents with positive controls and simple user-friendly protocols
- Validated in various cell/tissue types: HeLa, HL-60, HCT116, keratinocytes, and 3D liver microtissues

Mechanism of Action

The Hypoxia (Red) dye takes advantage of the nitroreductase activity present in hypoxic cells by converting the Nitro group to hydroxylamino (NHOH) and then to amino (NH_2), releasing the fluorescent probe. The Oxidative Stress Detection Reagent is a non-fluorescent, cell-permeable total ROS detection dye which reacts directly with a wide range of reactive species. The generated fluorescent products can be visualized using a wide-field fluorescence microscope equipped with standard fluorescein (490/525 nm) and Texas Red (596/670 nm) filters, confocal microscopy, or cytometrically using any flow cytometer equipped with a blue (488 nm) laser.

Multiplex, Real-time Analysis of Hypoxia and ROS

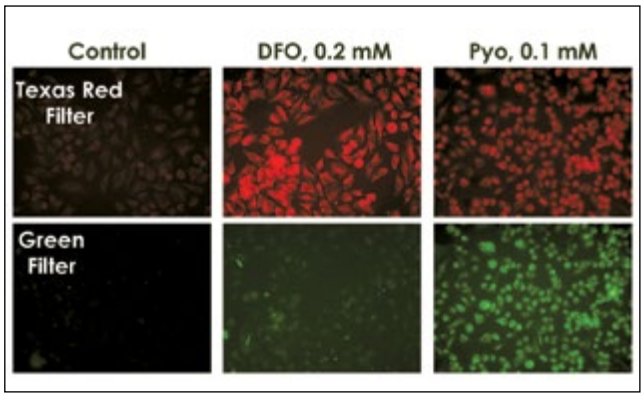


Figure 1: HeLa cells were subject to treatment. Bright red fluorescence of the Hypoxia probe is observed following its conversion by cellular nitroreductases under hypoxic conditions such as those induced chemically by treatment with the hypoxia-mimetic desferrioxamine (DFO).

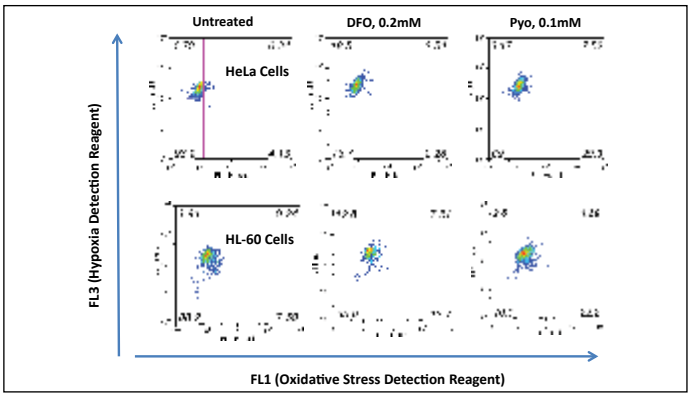


Figure 2: Detection of hypoxia and oxidative stress levels in cultured human HeLa and HL-60 cells. Cells were treated with hypoxia inducer (DFO) and ROS inducer (pyocyanin). Numbers in each quadrant reflects the percentage of cells (population). Results indicate that hypoxia and oxidative stress dye are specific.

Description	Cat. No.	Kit Components	
ROS-ID® Hypoxia/Oxidative Stress Detection Kit	10662-268 / 10136-016	Dyes, Pyocyanin, DFO	
Dyes Included in Kits	**Excitation/Emission**	**Platform**	**Cell Type**
Oxidative Stress Detection Reagent Hypoxia Dye	504/524nm ● 580/595nm ●		Live

Application Notes

1. Inhibition of Hypoxic Tumor Cells using a Three-Dimensional Spheroid Model
2. PMT and Image-Based Analysis of Hypoxia Induction using a 3D Spheroid Model

MULTIDRUG RESISTANCE

EFLUXX-ID® MULTIDRUG RESISTANCE ASSAY KITS GREEN (89165-922) / GOLD (89165-924)

Simple no-wash assays for simultaneous monitoring of all 3 major ABC transporter proteins – MDR, BCRP & MRP

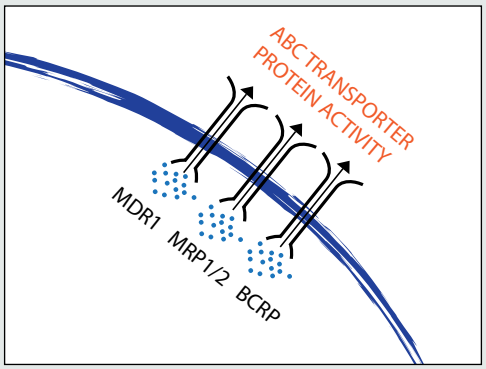
Drug resistance is a phenomenon mediated by up-regulation of a family of transmembrane ATP Binding Cassette (ABC) transporter proteins. Overexpression of ABC transporter proteins accelerates removal of toxic agents from the cell, for example the efflux of chemotherapeutic agents from tumor cells, or of antibiotics from resistant strains of bacteria.

The EFLUXX-ID® Multidrug Resistance Assay Kit allows for functional detection of all three clinically relevant ABC transporter proteins: MDR1 (p-glycoprotein), MRP1/2, and BCRP. The assay uses a hydrophobic, non-fluorescent compound that readily penetrates the cell membrane, where it is hydrolyzed to a hydrophilic fluorescent dye by intracellular esterases. The resulting fluorescent dye is trapped inside the cell unless it is actively pumped out by ABC transporter protein activity. Therefore, cells exhibiting drug resistance will have diminished fluorescence. EFLUXX-ID® assay is the only available kit for the simultaneous monitoring of all three major ABC reporter proteins with the ability to profile individual pump activity.

- Measures drug resistance related to activity of all three major ABC transporter proteins
- Single proprietary dye provides quantitative measurements of MDR activity in live cells expressed as MDR Activity Factor (MAF)
- Kit includes three known inhibitors specific for MDR1 (p-glycoprotein), MRP1/2, and BCRP
- Simple, no-wash protocol delivers results in 1 hour
- Available in green and gold fluorophores
- Comprehensive efflux detection assay for three major types of ABC transporters
- Detects BCRP activity not detected with Calcein AM
- Inhibitors included for profiling of specific pumps involved in drug resistance
- Two dye formats allows multiplexing with GFP-expressing cell lines or other CELLESTIAL® dyes

Mechanism of Action

The proprietary AM-ester form of the EFLUXX-ID® dye is a hydrophobic non-fluorescent compound that readily penetrates the cell membrane and is subsequently hydrolyzed inside of the cells by intracellular esterases. The resulting probe is a hydrophilic fluorescent dye that is trapped within the cell unless actively pumped out by an ABC transporter. The fluorescence signal of the dye generated within the cells thus depends upon the activity of the ABC transporters. The cells with highly active transporters will demonstrate lower fluorescence because of the active efflux of the probe from the cell. Application of specific inhibitors of the various ABC transporter proteins allows differentiation between the three common types of pumps.



Description	Cat. No.	Kit Components	
EFLUXX-ID® Multidrug Resistance Assay Kits Green/Gold	89165-922 / 89165-924	Dyes, MDR1 inhibitor, MRP inhibitor, BCRP inhibitor	
Dyes Included in Kits	**Excitation/Emission**	**Platform**	**Cell Type**
89165-922 EFLUXX-ID® Green Detection Reagent Propidium Iodide	490/514nm 535/617nm		Live
89165-924 EFLUXX-ID® Gold Detection Reagent Propidium Iodide	530/570nm 535/617nm		Live

MULTIDRUG RESISTANCE

Detect Activity of All Three Major ABC Transporter Proteins

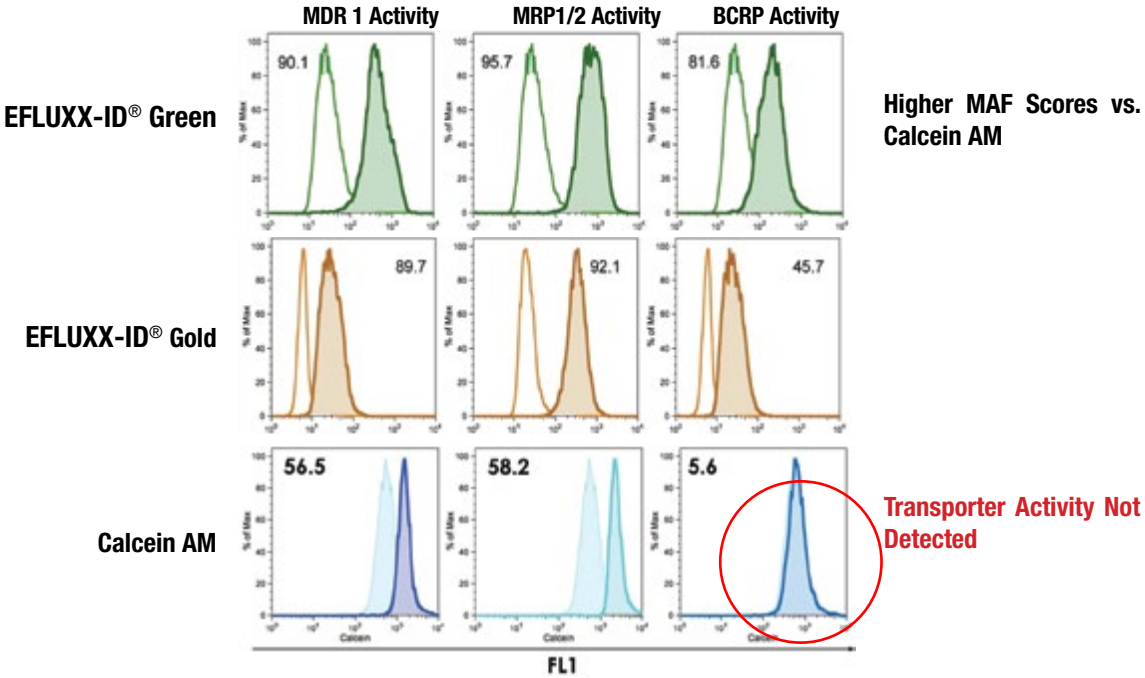


Figure 1: ABC transporter protein activity was evaluated in CHO K1 cells by flow cytometry using EFLUXX-ID® Green (top), Gold (middle), or Calcein AM (bottom) dyes. Treatment with specific inhibitors of ABC Transporter proteins (shaded histograms) induces retention of dye within cells relative to untreated cells (lined histograms). The difference in mean fluorescence intensity (MFI) is an indication of the corresponding protein activity, as shown by MAF scores [multidrug resistance activity factors], a quantitative measurement of multidrug resistance. Higher MAF scores are a result of superior specificity of EFLUXX-ID® dyes to specific inhibitors. Calcein AM (a common probe for MDR assays), is unable to detect BCRP activity.

Profiling of Specific ABC Transporter Protein Activity Using Known Inhibitors

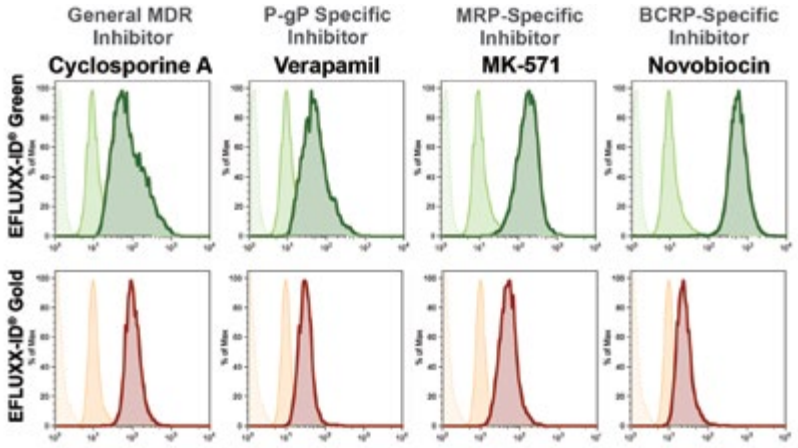


Figure 2: Profiling of ABC transporter activity by known inhibitors was assessed in CHO K1 cells using EFLUXX-ID® Green and EFLUXX-ID® Gold dyes. Cells were incubated for 5 min at 37°C with general MDR Inhibitor (far left column) or transporter-specific inhibitors included in the kit. Cells were then loaded with the indicated dye for 30 min at 37°C and immediately analyzed by flow cytometry. Inhibitors used: 5 µM Cyclosporin A (general MDR inhibitor); 20 µM Verapamil (specific P-gp inhibitor); 0.05 mM MK-571 (specific MRP inhibitor); 0.05 mM Novobiocin (specific BCRP inhibitor).

Citations

1. Lebedeva, I., Pande, P., Shen, D., Gatica, D. and Patton, W. "Sensitive and specific fluorescent probes for functional analysis of the three major types of mammalian ABC transporters" PLoS One. (2011); 6(7):e22429.

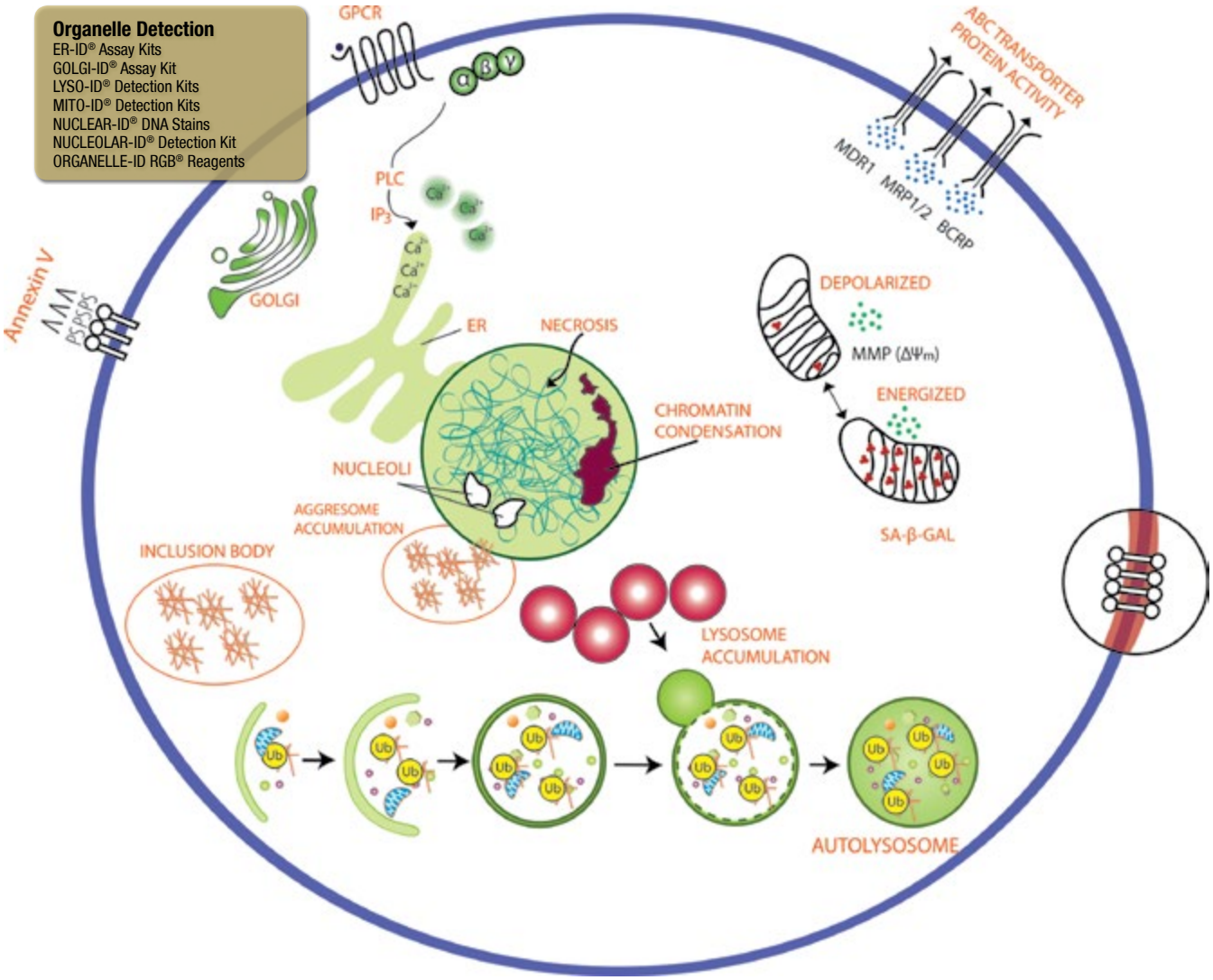
CELL STRUCTURE & ORGANELLE DETECTION

Enzo Life Sciences offers a wide array of products for researching and analyzing cell structure in both live and fixed platforms

Organelles provide key functions in support of cellular physiology, including intracellular protein degradation, lipid metabolism, ATP synthesis, protein post-translational modification and transmission of DNA in dividing daughter cells. Damaged or compromised organelles are often associated with the onset and progression of a variety of diseases, including cardiovascular, neurodegenerative, cancer, autoimmune and metabolic conditions. Organelle–targeting dyes are useful as counter-stains to aid in identifying cellular constituents, co-localizing specific proteins and targets of interest within subcellular compartments and monitoring morphological responses to chemical and environmental stresses. Selective degradation of mitochondria by mitophagy and morphological alterations in endoplasmic reticuli accompanying the unfolded protein response (UPR) are two examples wherein morphological changes in organelles can serve as indicators of cellular stress.

Our comprehensive panel of fluorescence-based assays, highlighting various structural features of different subcellular organelles, offers the prospect of identifying subtle *in vitro* responses that could serve as predictive surrogates for *in vivo* toxicity testing. Cell-based assays that monitor subcellular organelle morphology are increasingly becoming important to secondary screening campaigns because they can often provide higher value information than conventional assays of cell viability and death. The described assays enable investigation of organelle structure and function in living cells using a variety of instrumentation platforms including flow cytometers, microplate readers and fluorescence microscopes.

Organelle Detection
ER-ID® Assay Kits
GOLGI-ID® Assay Kit
LYSO-ID® Detection Kits
MITO-ID® Detection Kits
NUCLEAR-ID® DNA Stains
NUCLEOLAR-ID® Detection Kit
ORGANELLE-ID RGB® Reagents



NUCLEUS

NUCLEAR-ID® RED DNA STAIN (89166-036)

Brighter, cell permeable DNA stain that can be used for a wide range of applications

The NUCLEAR-ID® Red DNA Stain is a cell permeable dye, designed for use in a range of fluorescence detection technologies, in the discrimination of nucleated cells. It is resistant to photo-bleaching and is suitable for live-cell staining of nuclei. Also this dye provides a convenient approach for studying the induction and inhibition of cell cycle progression by flow cytometry. Potential applications of this reagent for live-cell studies are in the determination of cellular DNA content and cell cycle distribution, for the detection of variations in growth patterns, for monitoring apoptosis, and for evaluating tumor cell behavior and suppressor gene mechanisms.

- Brighter stain results in lowered concentration and cytotoxicity
- Far-red fluorescent specific DNA dye does not require UV laser source
- No photobleaching effect
- No RNase treatment is required
- GFP and FITC compatible

NUCLEAR-ID® Red DNA Stain Requires Lower Concentration than Competitor's Dye to Visualize DNA

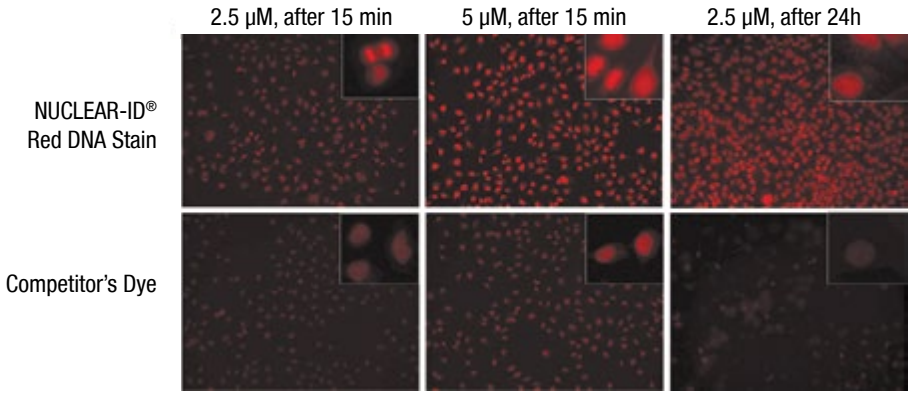


Figure 1: HeLa cells were stained with varying concentrations of either NUCLEAR-ID® Red DNA Stain or Competitor's dye and gently washed post-staining. The NUCLEAR-ID® Red DNA Stain requires lower concentration in live cell studies, resulting in lower costs and cytotoxicity.

Description	Cat. No.	Excitation/Emission	Platform	Cell Type
NUCLEAR-ID® Red DNA Stain	89166-036	568/637nm ●		Live, Fixed, Permeabilized

NUCLEAR-ID® BLUE DNA STAIN (GFP-CERTIFIED®) (10001-806)

Cell permeable DNA stain that can be used for a wide range of applications

- Stable and specific blue fluorescent DNA dye
- High purity
- No photobleaching effect
- No RNase treatment is required
- Multiplex with GFP and red dyes

Description	Cat. No.	Excitation/Emission	Platform	Cell Type
NUCLEAR-ID® Blue DNA Stain	10001-806	350/461nm ●		Live, Fixed, Permeabilized

NUCLEUS

NUCLEOLAR-ID® GREEN DETECTION KIT (89165-880)

Identify the nucleolus in live cells

The NUCLEOLAR-ID® Green Detection Kit contains a proprietary dye suitable for live-cell staining of nucleoli. The dye allows examination of nucleolar dynamic changes in intracellular distribution, trafficking and localization arising from biological processes such as the cell cycle and ribosome biogenesis. The kit is compatible with most fluorescence detection systems, including conventional and confocal fluorescence microscopes, as well as High Content Screening (HCS) platforms. Potential applications for this kit include monitoring impaired ribosome biogenesis, inhibition of transcription, cell cycle dynamics and cellular stress, as well as the distribution, trafficking and dynamics of nucleolar proteins, the distribution of viral proteins, and potentially as an aid in identifying cancer cells.

- Compatible with common live-cell nuclear counterstains (Hoechst 33342, Draq5™, Vybrant® DyeCycle™ Ruby, NUCLEAR-ID® Red dyes)
- High resistance to photobleaching and concentration quenching, ensuring strong, consistent fluorescence signal, even after extended viewing periods
- Stringently manufactured, to control and eliminate non-specific assay artifacts

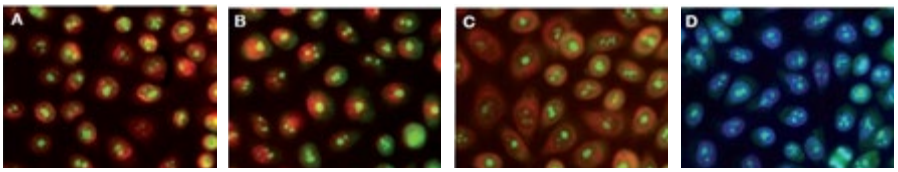


Figure 1: NUCLEOLAR-ID® Green dye stained live U-2 OS cells counterstained with NUCLEAR-ID® Red (A), Draq5™ (B), Vybrant® DyeCycle™ Ruby (C), and Hoechst 33342 (D). Nucleoli appear as green structures contrasted against the selected nuclear counterstain by fluorescence microscopy. As an RNA stain, the NUCLEOLAR-ID® Green dye displays some cytoplasmic staining as well, but the nuclear counterstain facilitates unambiguous identification of the nucleoli as green fluorescence signal within the confines of the highlighted nuclear fluorescence signal.

Description	Cat. No.	Kit Components	
NUCLEOLAR-ID® Green Detection Kit	89165-880	Dye, Actinomycin D, 10x assay buffer	
Dyes Included in Kits	**Excitation/Emission**	**Platform**	**Cell Type**
NUCLEOLAR-ID® Green Dye	450/481nm ●		Live

TOTAL-NUCLEAR-ID® GREEN/RED NUCLEOLAR/NUCLEAR DETECTION KIT (89165-874)

Simultaneous staining of both the nucleolus and the nucleus in live cells

The TOTAL-NUCLEAR-ID® Green/Red Nucleolar/Nuclear Detection Kit contains two proprietary dyes suitable for simultaneous live-cell staining of nucleoli and nuclei. This kit is specifically designed for visualizing nucleoli and nuclei in living cells. A control nucleolus perturbation agent, actinomycin D, is provided for monitoring changes in nucleolar dynamics.

- Validated for utility in live cell imaging applications, demonstrating appropriate response to treatment with well characterized nucleolus-perturbation agents

Description	Cat. No.	Kit Components		
TOTAL-NUCLEAR-ID® Green/Red Nucleolar/Nuclear Detection Kit	89165-874	Dyes, Actinomycin D, 10x assay buffer		
Dyes Included in Kit		**Excitation/Emission**	**Platform**	**Cell Type**
NUCLEOLAR-ID® Green Dye NUCLEAR-ID® Red Dye		450/481nm ● 568/637nm ●		Live

Detect the Nucleus and Nucleolus in Live Cells

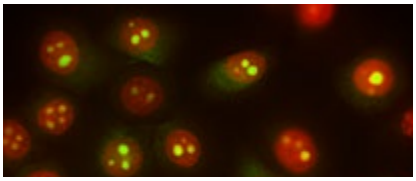


Figure 1: Live U-2 OS cells, stained with TOTAL-NUCLEAR-ID® Green/Red Nucleolar/Nuclear Detection Kit demonstrates staining of the nucleus (red) and nucleolus (yellow-green) via fluorescence microscopy. As an RNA stain, NUCLEOLAR-ID® Green dye does display some cytoplasmic staining as well, but NUCLEAR-ID® Red dye facilitates unambiguous identification of the nucleoli as green fluorescence signal within the confines of the highlighted red nuclear fluorescence signal.

ENDOPLASMIC RETICULUM

ER-ID® GREEN (89165-914) AND RED (89165-916) ASSAY KITS

Endoplasmic reticulum staining with minimal toxicity to cells

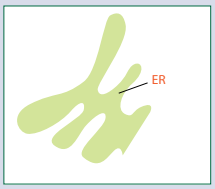
Fluorescence co-localization imaging with red fluorescent protein (RFP)-, orange fluorescent protein (OFP)- or green fluorescent proteins (GFP)-expressing cell lines, is a powerful approach for determining the targeting of molecules to intracellular compartments and for screening of their associations and interactions. However, to date, photoconversion of fluorescent dyes to other colors and metachromatic artifacts, wherein fluorescent dyes emit both in the red (or orange) and green regions of the spectrum, have led to spurious results in co-localization experiments. Additionally, many organelle-targeting probes photobleach rapidly, are subject to quenching upon concentration in organelles, are highly toxic, or only transiently associate with the target organelle, requiring imaging within a minute or two of dye addition.

ER-ID® Assay Kits contain cell-permeable, small molecule organic probes that spontaneously localize to live or fixed cells' endoplasmic reticula. Low micromolar concentrations of ER-ID® dyes are sufficient for staining mammalian cells, minimizing toxicity to the cells. ER-ID® dyes are resistant to photo-bleaching, concentration quenching and photoconversion, and can readily be used in combination with other common UV- and visible-light excitable organic fluorescent dyes and various labeled antibodies for multi-color imaging and detection applications.

- Novel endoplasmic reticulum-selective dye stains live, permeabilized or fixed cells
- Easily multiplexed with common fluorescent dyes and fluorescent proteins
- Highly resistant to photo-bleaching, concentration quenching and photo-conversion
- Low dye concentration minimizes toxicity to cells
- Validated with HeLa, Jurkat, and U-2 OS cell lines

Mechanism of Action

The dyes are cell-permeable and highly selective for the endoplasmic reticulum (ER). These dyes minimally stain mitochondria, unlike the conventional ER stain DiOC6(3), and staining at low concentrations does not appear to be toxic to cells. The precise mechanism of selective staining has not yet been determined but analogous dyes are thought to bind to the sulphonylurea receptors of ATP-sensitive K^+ channels, which are prominent on ER.



Counterstaining with Nuclear Dye Highlights Both Organelles

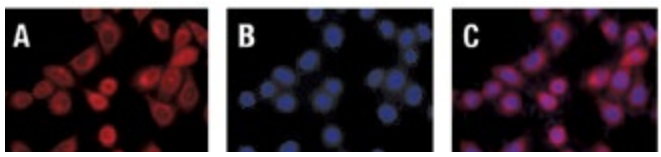


Figure 1: Live HeLa cells stained with ER-ID® Red dye (A), Hoechst 33342 nuclear stain (B) and resulting composite image (C).

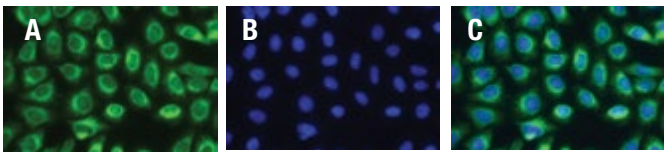


Figure 2: Live HeLa cells stained with ER-ID® Green dye (A), Hoechst 33342 nuclear stain (B) and resulting composite image (C).

Description	Cat. No.	Kit Components	
ER-ID® Green and Red Assay Kits	89165-914 / 89165-916	Dyes, 10x assay buffer	
Dyes Included in Kits	**Excitation/Emission**	**Platform**	**Cell Type**
89165-914 ER-ID® Green Detection Reagent Hoechst 33342 Nuclear Stain	 441/551nm ● 350/461nm ●		Live, Fixed, Permeabilized
89165-916 ER-ID® Red Detection Reagent Hoechst 33342 Nuclear Stain	 580/677nm ● 350/461nm ●		Live, Fixed, Permeabilized

GOLGI APPARATUS

GOLGI-ID® GREEN ASSAY KIT (89165-920)

Dependably localizes to the target organelle with minimal staining of ER

Fluorescence co-localization imaging in cells expressing blue, cyan, yellow, orange, or red fluorescent proteins (BFPs, CFPs, YFPs, OFPs, RFPs), is a powerful approach for determining the targeting of molecules to intracellular compartments and for screening of their associations and interactions. Many organelle-targeting probes photo-bleach rapidly, are subject to quenching upon concentration in organelles, are highly toxic or only transiently associate with the target organelle, requiring imaging within a minute or two of dye addition.

GOLGI-ID® Green dye is a new green-emitting, cell-permeable small molecule organic probe that spontaneously localizes to live or fixed Golgi apparatus. The dye can be readily used in combination with other common UV and visible light excitable organic fluorescent dyes and various fluorescent proteins in multicolor imaging and detection applications. The dye emits in the FITC region of the visible light spectrum, and is resistant to photo-bleaching, concentration quenching and photo-conversion. The kit should also be suitable for identifying Golgi body perturbing agents and therefore can be a useful tool for examining inhibition of the secretory pathway thus preventing movement of proteins and eventually, secretion out of the cell.

- Novel Golgi apparatus-selective dye stains live, permeabilized or fixed cells
- Easily multiplexed with common fluorescent dyes and fluorescent proteins
- Highly resistant to photo-bleaching, concentration quenching and photo-conversion
- Low dye concentration minimizes toxicity to cells
- Validated with HeLa, Jurkat, and U-2 OS cell lines

Mechanism of Action

GOLGI-ID® Green dye is a fluorescent lipid analog that is metabolized by cells and transported directly into the Golgi bodies where upon it interacts with endogenous lipids of that organelle. The dye is believed to be restricted to the luminal leaflet of the Golgi body membrane. The fluorescent lipid appears to preferentially stain the trans-Golgi stacks of the organelle. Both living and fixed cells are stainable. However, fixation protocols that extract or modify cellular lipids must be avoided.

Co-localization Facilitates Studies of Intracellular Protein Trafficking via the Cellular Secretory Pathway

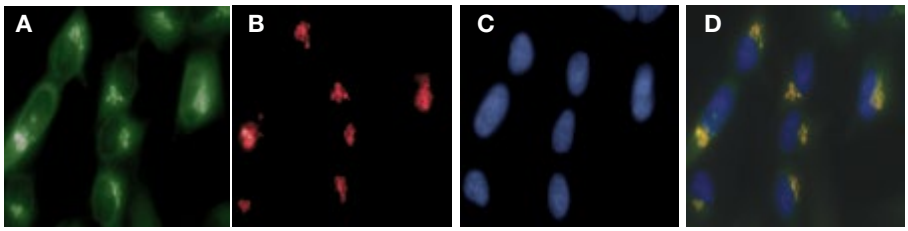


Figure 1: Co-localization Imaging with RFP-tagged Proteins. GOLGI-ID® Green dye co-localizes with N-acetylgalactosaminyltransferase-2 fused to red fluorescent protein (RFP). (A) GOLGI-ID® Green dye, (B) RFP-N-acetylgalactosaminyltransferase-2, (C) Hoechst 33342 nuclear stain, (D) composite image.

Description	Cat. No.	Kit Components	
GOLGI-ID® Green Assay Kit	89165-920	Dyes, 10x assay buffer 1, 50x assay buffer 2	
Dyes Included in Kits	**Excitation/Emission**	**Platform**	**Cell Type**
GOLGI-ID® Green Detection Reagent Hoechst 33342 Nuclear Stain	473/534nm ● 350/461nm ●		Live, Fixed

LYSOSOMES

LYSO-ID® RED (10662-272 / 89165-872) / GREEN (89165-930 / 10662-270) DETECTION KIT

Cationic amphiphilic tracer dye monitors dysfunction of the lysosomal degradation pathway by rapid and selective acidic organelle staining

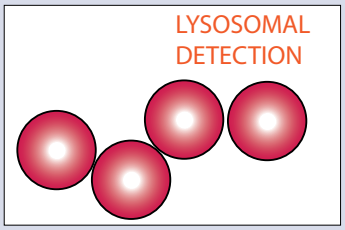
The identification of cytotoxic effects is a critical element in the pre-clinical small molecule drug discovery process. In vitro cell-based assays are typically used early in the process following screening of target molecules. Cytotoxic effects of drug molecules are often first observed as perturbations of normal cellular organelle functionality. As an example, lysosome perturbation as a result of ion trapping of amine-containing compounds has been demonstrated to cause the formation of autophagolysosomes and autophagic cytopathology.

LYSO-ID® Detection Reagents are fluorescent dyes that monitors dysfunction of lysosomal degradation using a drug-like cationic amphiphilic tracer (CAT) dye that rapidly and selectively stains acidic organelles. An increase in signal is indicative of an increase in the number or size of cellular lysosomes and lysosome-derived vacuoles allowing monitoring of the accumulation of lysosomes and lysosome-like structures in live cells.

- Novel acidic organelle-selective dyes are suitable for live cell imaging of lysosomes
- LYSO-ID® Red dye easily multiplexes with common fluorescent dyes (coumarin, FITC, Cyanine-3) and fluorescent proteins (BFP, CFP, GFP, YFP)
- LYSO-ID® Green dye easily multiplexes with cell lines expressing BFP, CFP, OFP and RFP
- Highly resistant to photobleaching and concentration quenching, ensuring strong, consistent fluorescence signal, even after extended viewing time
- Suitable for both short-term and long-term tracking studies
- Highly resistant to photobleaching and concentration quenching, ensuring strong, consistent fluorescence signal, even after extended viewing time

Mechanism of Action

LYSO-ID® Red Detection Reagents are cationic amphiphilic tracer (CAT) dyes that rapidly partition into cells in a similar manner as drugs that induce phospholipidosis. The dye was created through careful selection of titratable groups on the probe, to enable labeling to be expanded into lamellar inclusion bodies of cells pretreated with weakly basic cell-permeant compounds, such as the antimalarial drug chloroquine. Besides lysosomes themselves, the probe can be employed for highlighting lysosome-like organelles under conditions wherein cells produce vacuoles that contain most of the degradative enzymes of the lysosome but are not as acidic as the parent organelle.



Drug-Induced Vesicle and Volume Increase Detected by LYSO-ID® Red Dye

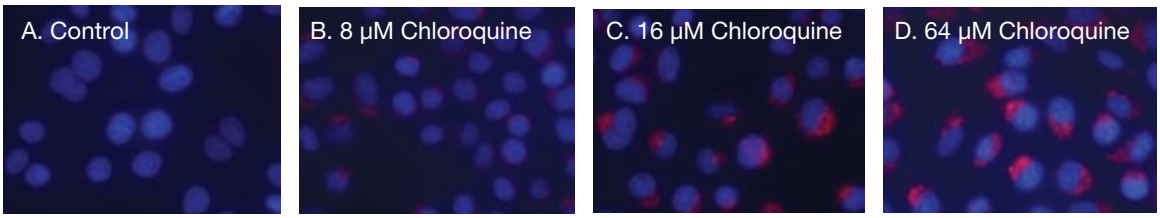


Figure 1: LYSO-ID® Red dye is a cell-permeant fluorescent probe that selectively associates with lysosomes and other acidic organelles (A). U-2 OS cells were pre-treated for 18 hours with weakly basic, cell-permeant chloroquine. An increase in lysosome-like vesicle number and volume is observed (shown in B-D). This confirms LYSO-ID® Red dye partitions with the lysosomal compartment. Nuclei are counter-stained with Hoechst 33342 in the images.

LYSOSOMES

Drug-Induced Vesicle and Volume Increase Detected by LYSO-ID® Red Dye

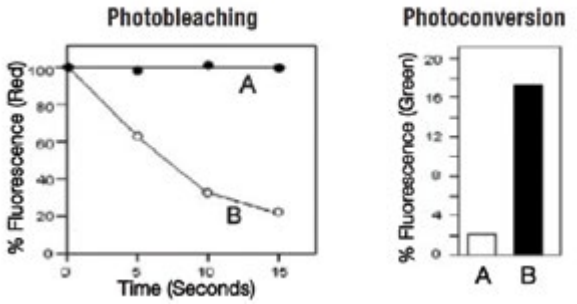


Figure 2: LYSO-ID® Red dye (A) is highly photostable. Unlike the leading competitor's lysosomal stain (B), it does not photo-convert to a green fluorophore, making the probe especially useful when combined with GFP constructs and for other multicolor cell analysis applications.

Drug-Induced Vacuole Number and Volume Increase Detected by LYSO-ID® Green Dye

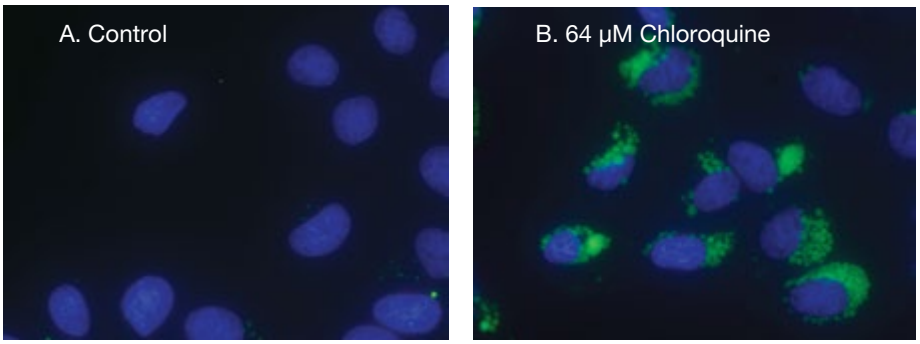


Figure 3: LYSO-ID® Green dye is a cell-permeant fluorescent probe that selectively associates with lysosomes and other acidic organelles (A). HeLa cells were pre-treated for 20 hours with weakly basic cell-permeant compound, chloroquine, show dramatic increases in lysosome-like vesicle number and volume (B). Nuclei are counter-stained with Hoechst 33342 in the images.

Description	Cat. No.	Kit Components	
LYSO-ID® Red / Green Detection Kits	10662-272 / 89165-872 89165-930 / 10662-270	Dyes, Chloroquine, 10x assay buffer	
Dyes Included in Kits	**Excitation/Emission**	**Platform**	**Cell Type**
10662-272 / 89165-872 LYSO-ID® Red Detection Reagent Hoechst 33342 Nuclear Stain	 568/667nm ● 350/461nm ●		Live
89165-930 / 10662-270 LYSO-ID® Green Detection Reagent Hoechst 33342 Nuclear Stain	 481/544nm ● 350/461nm ●		Live

Citations

1. Akbar MA, Tracy C, Kahr WH, Krämer H. The full-of-bacteria gene is required for phagosome maturation during immune defense in Drosophila. J Cell Biol. (2011) 192(3):383-90.
2. Coleman J., Xiang Y., Pande P., Shen D., Gatica D., and Patton W. "A live-cell fluorescence microplate assay suitable for monitoring vacuolation arising from drug or toxic agent treatment." J Biomol Screen. (2010) 15(4) 398-405.
3. Kwok AS, et.al. HspB8 Mutation Causing Hereditary Distal Motor Neuropathy Impairs Lysosomal Delivery of Autophagosomes. J Neurochem. 2011 Oct 10. doi: 10.1111/j.1471-4159 2011 07521.x. [Epub ahead of print]

MITOCHONDRIA

MITO-ID® RED (10662-276 / 89165-876) / MITO-ID® GREEN (89165-908 / 10551-282) DETECTION KITS

MITO-ID® dyes are photo-stable, non-toxic, and selectively stain regardless of mitochondrial membrane potential

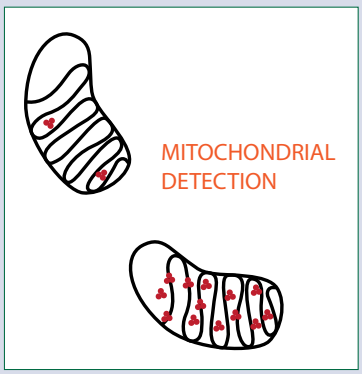
Impairment of mitochondrial function is increasingly implicated in the etiology of drug-induced toxicity. Mitochondria produce >90% of the cellular energy requirements in the form of adenosine triphosphate (ATP) via oxidative phosphorylation. The mitochondrion is a key regulator of the metabolic activity of the cell, and is also an important organelle in both production and degradation of free radicals. It is thought that higher mitochodrial copy number (or higher mitochondrial mass) is protective for the cell.

MITO-ID® Red and Green dyes highlight mitochondria regardless of their energetic state (mitochondrial membrane potential). The dyes are compatible with most fluorescence detection systems, including conventional and confocal fluorescence microscopes, as well as High Content Screening (HCS) platforms. The kits are useful for assessing mitochondrial morphology changes and estimating mitochondrial mass. An important application of MITO-ID® Red and Green dyes is in fluorescence co-localization imaging with fluorescent proteins. This is a powerful approach for determining the targeting of molecules to intracellular compartments, and for screening of associations and interactions between these molecules. Additionally, the kit is suitable for use with live or post-fixed cells in conjunction with fluorescent probes, such as labeled antibodies.

- Potent mitochondria-selective dye suitable for live, detergent-permeabilized and fixed-cell staining
- Easily multiplexed with common fluorescent probes
- Highly resistant to photobleaching and concentration quenching, ensuring strong, consistent fluorescence signal, even after extended viewing periods
- Non-toxic to most cell lines
- Highlights mitochondria regardless of the organelle's membrane potential status

Mechanism of Action

MITO-ID® dyes target a component of the inner mitochondrial membrane in mammalian cells—cardiolipin. Cardiolipin represents approximately 20% of the total lipid composition in the inner mitochondrial membrane, where it is essential for the optimal function of numerous enzymes that are involved in mitochondrial energy metabolism. Accumulation of these probes in mitochondria is largely independent of the mitochondrial membrane potential.



Citations

1. Cottet-Rousselle C, Ronot X, Leverve X, Mayol JF. Cytometric assessment of mitochondria using fluorescent probes. Cytometry A. 2011 Jun;79(6):405-25.
2. Trisciuoglio D, Ragazzoni Y, Pelosi A, Desideri M, Carradori S, Gabellini C, Maresca G, Nescatelli R, Secci D, Bolasco A, Bizzarri B, Cavaliere C, D'Agnano I, Filetici P, Ricci-Vitiani L, Rizzo MG, Del Bufalo D. CPTH6, a thiazole-derivative, induces histone hypoacetylation and apoptosis in human leukemia cells. Clin Cancer Res. 2012 Jan 15;18(2):475-486.

MITOCHONDRIA

Co-localization with GFP-expressing Cell Line Shows MITO-ID® Dye Specificity to Mitochondria

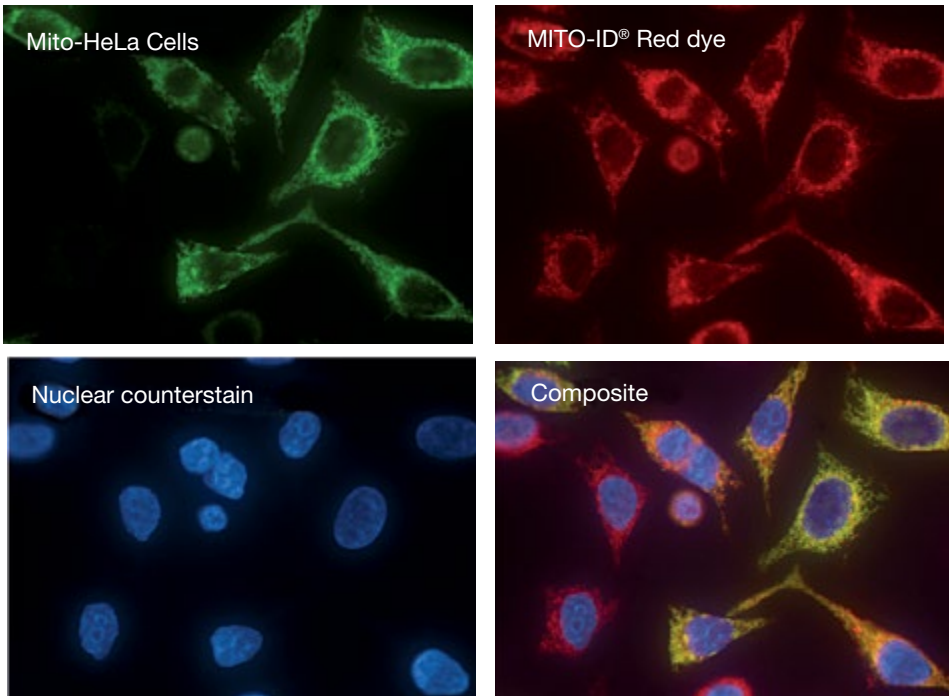


Figure 1: HeLa-TurboGreen-mitochondria cell line (expressing GFP-cytochrome c oxidase) stained with MITO-ID® (Red) and Hoechst 33342 (Blue) dyes. MITO-ID® Red co-localizes with the EGFP-cytochrome c oxidase signal (yellow or orange signal), demonstrating selectivity for mitochondria. Note that mitochondria in cells no longer expressing the GFP-tagged protein, or reduced amounts, appear red in the composite image.

MITO-ID® Dyes Selectively Stain Regardless of Mitochondrial Membrane Potential

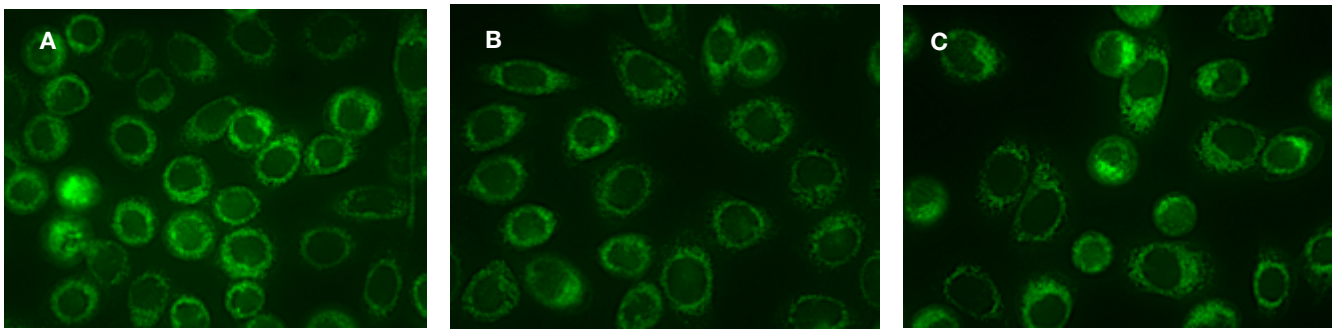


Figure 2: The MITO-ID® Green dye selectively stains mitochondria of living cells (A) and is relatively insensitive to mitochondrial membrane potential uncouplers of phosphorylation, such as CCCP (carbonyl cyanide 3-chlorophenylhydrazone) (B) as well as ion-channel perturbing drugs, such as valinomycin (C).

Description	Cat. No.	Kit Components	
MITO-ID® Red / Green Detection Kits	10662-276 / 89165-876 89165-908 / 10551-282	Dyes, 10x assay buffer	
Dyes Included in Kits	**Excitation/Emission**	**Platform**	**Cell Type**
10662-276 / 89165-876 MITO-ID® Red Detection Reagent Hoechst 33342 Nuclear Stain	558/690nm ● 350/461nm ●		Live, Fixed, Permeabilized
89165-908 / 10551-282 MITO-ID® Green Detection Reagent Hoechst 33342 Nuclear Stain	460/560nm ● 350/461nm ●		Live, Fixed, Permeabilized

MULTIPLE ORGANELLES

ORGANELLE-ID RGB® III ASSAY KIT (89165-928)

Mixture of fluorescent dyes for detection of endoplasmic reticulum, Golgi apparatus and nucleus

The endoplasmic reticulum (ER) and Golgi apparatus (GA) are the primary organelles responsible for the proper sorting of lipids and proteins by cells. After synthesis, folding and quality control, the lipid and protein cargo exit from the ER and enter the GA through the ER-Golgi interface. Within the ER-Golgi interface, COPII-mediated concentration of membrane and soluble cargo occurs and various post-translational modifications take place prior to delivery to the GA. Transient ER-Golgi connections are likely to serve a role in the diffusion of cargo proteins as well as the recycling of organelle resident proteins. The structure and functions of the various compartments along the secretory pathway are considered complicated, so simple visualization and unambiguous categorization of the ER and GA in living cells is a valuable tool.

The GOLGI-ID® Green dye component of the ORGANELLE-ID RGB® III Detection Reagent is faithfully localized to the Golgi apparatus, with minimal staining of the endoplasmic reticulum. The ER-ID® Red dye component of the reagent stains the endoplasmic reticulum with high fidelity and is specifically designed for use with green fluorescing probes. ORGANELLE-ID RGB® III Assay Kit is validated with human cervical carcinoma cell line-HeLa, human T-lymphocyte cell line-Jurkat, canine kidney cell line-MDCK, and human bone osteosarcoma epithelial cell line-U2 OS. The kit is suitable for identifying Golgi body and endoplasmic reticulum perturbing agents and thus can be a useful tool for examining the transport and recycling of molecules from the GA to ER in cellular secretory pathways.

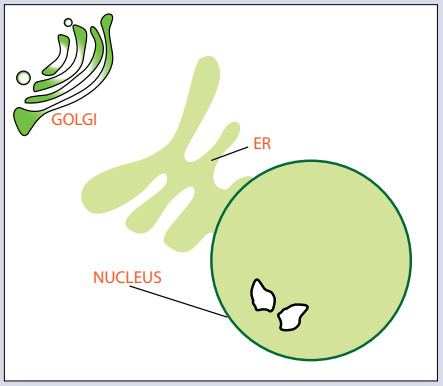
- Simple one-tube format containing all three dyes
- Highly resistant to photo-bleaching, concentration quenching and photoconversion
- Identify modulators of secretory membrane traffic and organelle integrity
- Simple visualization and categorization of the ER and GA dynamics in living cells

Mechanism of Action

Nucleus Blue Hoechst 33342 dye: cell-permeant nuclear counterstain that emits blue fluorescence when bound to the adenine/thymine-rich regions in the minor groove of deoxyribonucleic acid (DNA).

ER-ID® Red dye: The dye is cell-permeable and highly selective for the endoplasmic reticulum (ER). The dye minimally stains mitochondria, unlike the conventional ER stain DiOC6(3), and staining at low concentrations does not appear to be toxic to cells. The precise mechanism of selective staining has not yet been determined but analogous dyes are thought to bind to the sulphonylurea receptors of ATP-sensitive K+ channels, which are prominent on ER.

GOLGI-ID® Green dye: A fluorescent lipid analog that is metabolized by cells and transported directly into the Golgi bodies where upon it interacts with endogenous lipids of that organelle. The dye is believed to be restricted to the luminal leaflet of the Golgi body membrane. The fluorescent lipid appears to preferentially stain the trans-Golgi stacks of the organelle. Both living and fixed cells are stainable. However, fixation protocols that extract or modify cellular lipids must be avoided.



MULTIPLE ORGANELLES

Selectivity of Organelle Dyes

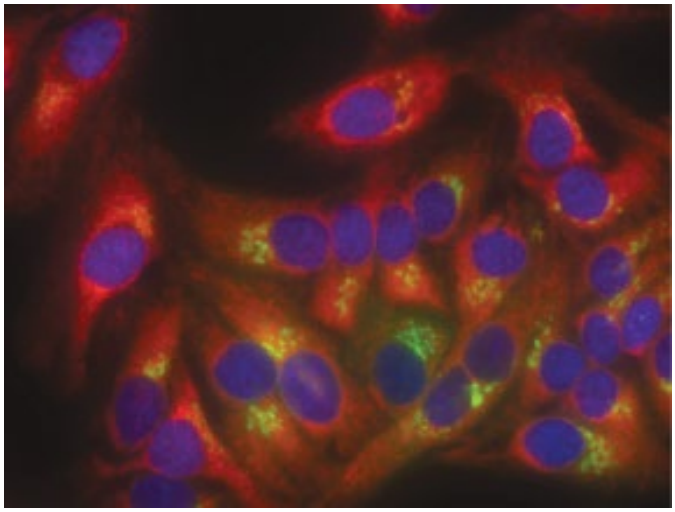


Figure 1: ORGANELLE-ID RGB® III staining in MDCK cells (89165-928). The selectivity of the ER-ID® Red dye, GOLGI-ID® Green dye and the Hoechst 33342 dye is evident.

3D Imaging of ER (red) and Golgi body (green) Displayed Relative to Nucleus (blue)

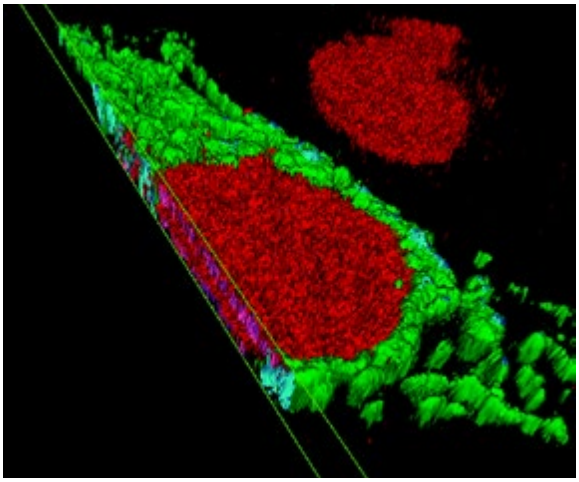


Figure 2: A three-dimensional reconstruction of the spatial relationship between the Golgi body and the endoplasmic reticulum using a structured illumination method was accomplished with the Apotome device from Carl Zeiss, Inc. This imaging hardware enabled creation of optical sections through the cell using a conventional wide field fluorescence microscope, for improved resolution along the optical axis. The optical sections were then used to create a 3-D reconstruction of the endoplasmic reticulum (red) and Golgi body (green), enabling display in their proper spatial context relative to the nucleus (blue).

Description	Cat. No.	Kit Components	Dyes Included	Ex/Em	Platform	Cell Type
ORGANELLE-ID® RGB III Assay Kit	89165-928	Dye Cocktail, 10x assay buffer 1, 50x assay buffer 2	ER-ID® Red GOLGI-ID® Green Nucleus Blue Hoechst 33342	580/677nm 473/543nm 350/461nm		Live
ORGANELLE-ID® RGB Reagent I	89166-070	Dye Cocktail	LYSO-ID® Red MITO-ID® Green Nucleus Blue Hoechst 33342	568/667nm 460/560nm 350/461nm		Live
ORGANELLE-ID® RGB Reagent II	89166-072	Dye Cocktail	LYSO-ID® Red ER-ID® Green Nucleus Blue Hoechst 33342	568/667nm 460/560nm 350/461nm		Live
ORGANELLE-ID® RGB Reagent IV	10136-044	Dye Cocktail	ER-ID® Red LYSO-ID® Green Nucleus Blue Hoechst 33342	568/667nm 481/544nm 350/461nm		Live

GOLD STANDARD DYES

Common Fluorescent Probes for Cellular Analysis			
Dyes	**Cat. No.**	**CELLESTIAL® Product**	**Ex/Em**
Amine-Reactive	89165-966	5-Carboxyfluorescein (Ultra Pure)	492/581nm
	89166-038	5-FITC (Ultra Pure)	494/520nm
	89166-040	5(6)-TRITC (Ultra Pure)	543/571nm
	89165-762	Cyanine 3-NHS Ester Pack	553/570nm
	89165-764	Cyanine 5-NHS Ester Pack	650/664nm
	89166-046	Dansyl chloride (Ultra Pure)	372/557nm
	89166-048	NBD-Cl (Ultra Pure)	337/512nm
Amyloid Detection	89166-056	Congo Red (Ultra Pure)	497/614nm
Calcium Indicators	89165-934	Calcein AM (Ultra Pure)	495/515nm
	89165-938	Fluo-3 AM (Ultra Pure)	506/526nm
	89165-970	Coelenterazine (Ultra Pure)	429/466nm
	89165-942	FURA-2 AM (Ultra Pure)	370/476nm
	89165-944	FURA-2 (Ultra Pure)	363/512nm
	89165-946	INDO-1 AM (Ultra Pure)	346/475nm
	89165-950	Rhod-2 AM (Ultra Pure)	549/578nm
	89165-956	Quin-2 (Ultra Pure)	346/475nm
Chloride Indicators	89165-986	Lucigenin (Ultra Pure)	455/505nm
	89165-990	MQAE (Ultra Pure)	350/460nm
Lipid Detection	89166-054	Nile Red (Ultra Pure)	552/636nm
Membrane Potential Detectors	89165-994	Di-2-ANEPEQ	517/721nm
	89165-996	Di-8-ANEPPS	498/713nm
	89165-998	DiBAC4(3) (Ultra Pure)	493/516nm
	89166-002	DiI1(5) iodide (Ultra Pure)	638/658nm

GOLD STANDARD DYES

Common Fluorescent Probes for Cellular Analysis			
Dyes	**Cat. No.**	**CELLESTIAL® Product**	**Ex/Em**
Mitochondrial Detection	89166-010	Dihydrorhodamine 123 (Ultra Pure)	507/529nm
	89166-012	DiOC6(3) iodide (Ultra Pure)	482/504nm
	89166-014	JC-1 (Ultra Pure)	515/529nm
	89166-018	NAO [Nonyl Acridine Orange] (Ultra Pure)	495/519nm
	89166-020	Rhodamine 123 (Ultra Pure)	507/529nm
	89166-022	TMRE (Ultra Pure)	549/574nm
Neuronal Detection	89166-008	Hydroxystilbamidine (Ultra Pure) (FLUORO-GOLD™ alternative)	385/536nm
	89166-004	MM 1-43 (FM® 1-43 alternative)	510/626nm
	89166-006	MM 1-64 (FM® 4-64 alternative)	558/734nm
Nuclear Detection	89166-034	Acridine Orange (Ultra Pure)	500/525nm
	89166-032	DAPI (Ultra Pure)	358/461nm
	89166-028	Hoechst 33258 (Ultra Pure)	352/461nm
	89166-026	Hoechst 33342 (Ultra Pure)	350/461nm
	89166-030	Propidium Iodide (Ultra Pure)	535/617nm
pH Indicator	89165-974	BCECF AM (Ultra Pure)	505/520nm
Reactive Oxygen Detection	89165-976	5(6)-CFCFDA (Ultra Pure)	494/521nm
	89165-978	5(6)-CFDA (Ultra Pure)	494/521nm
	89166-024	Luminol (Ultra Pure)	355/411nm
	89165-870	Red Hydrogen Peroxide Assay Kit	570/585nm
Thiol-Reactive	89166-052	Fluorescein-5-maleimide (Ultra Pure)	493/515nm
	89166-050	Monobromobimane [mBBR] (Ultra Pure)	395/490nm
Zinc Ion Indicators	89165-984	TSQ (Ultra Pure)	344/385nm
	89165-980	Zinquin ethyl ester (Ultra Pure)	368/490nm
	89165-982	Zinquin free acid (Ultra Pure)	368/490nm

0217 Lit. No. 051977W