GE Healthcare

Cell Proliferation Flourescence Kit

Product Booklet

Codes: 25-9001-89

Page Finder

1. Legal

http://www.gehealthcare.com/lifesciences

GE Healthcare UK Limited.
Amersham Place, Little Chalfont,
Buckinghamshire, HP7 9NA UK

2. Handling

2.1 Safety warnings and precautions

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Use of this product for *in vivo* animal investigations and experiments is the sole responsibility of the user. User must obtain approval from the relevant authority for such experiments and must ensure compliance with all international, national and local regulations.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2 Storage and stability

Store at 2–8°C

2.3 Expiry date

The expiry date is stated on the package and will be at least 4 weeks from the date of dispatch.

3. Components and other materials required

3.1 Components

1. Labelling reagent
The labelling reagent is supplied as a concentrated aqueous solution of 5-bromo-2'-deoxyuridine and 5-fluoro-2'-deoxyuridine (10:1 ratio). 250 µl

2. Nuclease
The nuclease for DNA denaturation is supplied in freeze dried Tris buffered saline containing bovine serum albumin, magnesium chloride and a stabilizer. 26 ml when reconstituted.

3. Anti-5-bromo-2'-deoxyuridine monoclonal antibody
The monoclonal antibody is supplied in Tris buffered saline containing bovine serum albumin, magnesium chloride and preservative. The monoclonal antibody is of murine origin, subclass IgG2a. 265 µl.

4. Cy™5 labelled goat anti mouse IgG (H+L)
The Cy 5 labelled goat anti mouse IgG is supplied in freeze dried phosphate buffered saline containing bovine serum albumin and 0.1% (w/v) Sodium Azide. 275 µl when reconstituted.

3.2 Other materials and equipment required

The following materials and equipment are required but not supplied:

- Pipettes or pipetting equipment with disposable sterile polypropylene tips.
- Microscope (phase contrast).
- Haemocytometer.
- Tissue culture incubator.

- Tissue culture medium.
- Distilled or deionized water.
- Sterile, disposable universal containers or sterilin pots.
- Refrigerator at 2–8°C.
- Multi-channel pipettor.
- Phosphate buffered saline (see reagent preparation section).
- Formalin solution (e.g., Sigma HT50-1-2, 4% formalin solution).
- Triton X-100 non-ionic detergent.
- Hoechst nuclear dye, for microscopy assays (e.g., Molecular Probes 33342).
- Black, clear-bottom, flat-bottomed 96-well microplate, tissue culture grade that is compatible with detection instrumentation (e.g., for detection using GE Healthcares' IN Cell Analyzer 1000, black 96 well clear bottom plates are recommended, Greiner 655090).
- Detection instrumentation, e.g.,
  Fluorescence plate reader.
  Fluorescence microscope.

4. Description

Traditionally, the study of cell proliferation has involved the use of [^{3}H]thymidine to allow monitoring of DNA synthesis in individual cells by autoradiography, and hence identify proliferating cells or cell populations.

More recently, alternative rapid non-radioactive techniques have been developed in which a thymidine analogue, 5-bromo-2'-deoxyuridine (BrdU), is incorporated into replicating DNA and subsequently localized using a specific monoclonal antibody (1, 2) . To increase the sensitivity of this technique, cells to be labelled with BrdU are simultaneously exposed to 5-fluoro-2'-deoxyuridine, an inhibitor of thymidilate synthetase (9), which increases BrdU incorporation by lowering competition by endogenous thymidine.

One drawback of this approach to detecting cell proliferation has been the need to denature cellular DNA to allow antibody access to BrdU. This has usually been achieved by treatment with acid or alkali which may give inconsistent results and adversely affects cell and tissue morphology.

GE Healthcares' cell proliferation kit avoids these problems by using nuclease digestion of DNA to allow antibody access. This process is achieved under mild conditions, simultaneously with antibody incubation (4, 8). Detection of bound antibody is achieved using a Cy 5 labelled antibody to mouse immunoglobulin, giving a fluorescent signal at sites of BrdU incorporation.

Advantages of the assay system include:

- Cellular morphology is preserved allowing multiplexing with other assays.
- Red-shifted dye, minimizes interference from autofluorescence.
- Compatible with GE Healthcares' IN Cell Analyzer 3000 and IN Cell Analyzer 1000.

- Can be used in conjunction with GE Healthcares' GFP based G2M Cell Cycle Phase Marker Assay to determine cell cycle position throughout the cell cycle.

5. Detection Instrumentation

The following detection systems are all suitable for analysis of the cell proliferation fluorescence assay:

- Fluorescent microscope/automated sub-cellular imager, e.g., IN Cell Analyzer 1000 and IN Cell Analyzer 3000 may be used for detection. The Object Intensity and Cell Cycle Trafficking Analysis modules may be used for image analysis (GE Healthcare).

IN Cell Analyzer 1000 and IN Cell Analyzer 3000

The IN Cell Analyzer 3000 is a line-scanning, laser-based, confocal imaging system with three high-speed CCD cameras. It has been developed specifically for performing information-rich cellular assays very rapidly and at high resolution, enabling high-throughput and high-content testing of drug compounds.

The IN Cell Analyzer 1000 is a bench top automated microscope system designed for imaging sub-cellular end point assays. The systems core components are a Nikon microscope, xenon lamp and high resolution CCD camera.

There are a number of analysis modules available with both of the systems, as well as the capability to export images and data into other commercially available analysis packages. The Object Intensity Analysis Module for the IN Cell Analyzer 1000 and IN Cell Analyzer 3000 can be used to measure nuclear-associated Cy 5 fluorescence.

Advantages of using the IN Cell Analyzer 1000 or IN Cell Analyzer 3000

- The Object Intensity analysis module measures the fluorescence intensity within each individual nucleus, therefore background fluorescence is minimal.
- When measuring cell proliferation using a plate reader, cell loss will result in a reduction in signal. Microscopic examination of cells

may confirm whether a reduction in signal is due to inhibition of cell proliferation, or cell death.

A 'Nuclei Count' (showing the number of cells analyzed per analysis field) is included in the data output from the Object Intensity Analysis Module. This aids monitoring of cell loss on a high-throughput scale and can be used to determine cytostatic / cytotoxic levels of drug.

- Plate reader,
  e.g., A Tecan Ultra fluorescence plate reader with filter sets optimized for cyanine dyes.

The assay can be used on fluorescence plate readers with an appropriate filter set (Cy5 Excitation maximum 646 nm, Emission maximum 664 nm).

Note: In our experience we have found other plate readers to be less sensitive. We recommend increasing the BrdU incubation time to increase the assay sensitivity that can be achieved with such instruments. Longer incubation periods will determine the replicating population but not the fraction of cell in S-phase.

6. Critical Parameters

The following points are critical.

- Allow all reagents to reach room temperature before use.
- Freeze dried reagents must be fully reconstituted before use.
- Gently mix all reagents prior to use.
- Avoid excess foaming of reagents.
- To maximize sensitivity and minimize non-specific staining, incubation times must be carried out exactly. If more than one plate is being assayed, each plate must be timed individually.
- Keep the wells covered with lids except when adding reagents and reading.
- For optimum data quality, samples should be assayed in triplicate.
- Prior to fixing cells, the assay should be performed under sterile conditions.

7. Assay Procedure – 96 well plates.

7.1 Reagent preparation

1. Phosphate buffered saline

11.5 g Na_2HPO_4

2.96 g NaH_2PO_4 .$2H_20$

5.84 g NaCl

in 1 litre of distilled water, (or 500 ml of distilled water for a 2X PBS solution).

Alternative similar formulations and commercially available concentrates, tablets etc., may be used.

2. Fixing solution (1% formalin, 0.1% Triton in PBS)

25 ml distilled water

25 ml 4% formalin solution

50 ml 2X PBS

100 µl Triton X-100

3. Labelling reagent

Dilute the BrdU labelling reagent (25-9001-89V2) 1:250 with sterile culture media, e.g., for 500 assays, dilute 200 µl in 50 ml sterile culture media. This will give a final dilution of 1:500 in the well. Concentrated labelling reagent is stable on storage at 2–8ºC for at least 3 months, diluted reagent should be discarded after use.

4. Anti-5-bromo-2'-deoxyuridine/nuclease

Reconstitute the vial of freeze dried nuclease (25-9001-89V1) with 26 ml of distilled/ deionized water. Add 260 µl of anti-5-bromo-2'-deoxyuridine monoclonal antibody (25-9001-89V3) and gently mix (do not vortex mix).

The undiluted monoclonal antibody and freeze dried nuclease are stable for at least 3 months when stored at 2–8ºC.

Once reconstituted the anti-5-bromo-2'-deoxyuridine/nuclease reagent must be stored at 2–8°C and used within 4 weeks.

5. Cy 5 labelled goat anti mouse IgG

Reconstitute the vial of freeze dried Cy 5 labelled goat anti mouse IgG (25-9001-89V4) with 275 µl distilled/ deionized water and gently mix to obtain a solution at 1 mg/ml. This can be stored at -20°C in suitable aliquots for at least 6 months. Avoid repeated freeze-thaw cycles.

Once reconstituted, further dilute the Cy 5 labelled goat anti mouse IgG 1:100 with PBS to obtain a solution at 10 µg/ml, (e.g., for 500 assays, dilute 250 µl in 25 ml PBS). Do not prepare more reagent than required for one experiment.

7.2 Assay Protocol – 96 well plates.

Note: Equilibrate all reagents to room temperature and gently mix before use.

1. Controls

To ensure confidence in interpretation of experimental results always include two sets of controls as part of each experiment.

Assay blank:

This should be included in each experiment. The assay blank provides information on non-specific binding of BrdU, anti-BrdU and Cy 5 labelled goat anti mouse to the microplate. For assays read on a plate reader or macro-imager, the value obtained from this control should be subtracted from all other values.

Non-specific binding control:

The non-specific binding control provides information on the binding of the anti-BrdU and the Cy 5 labelled goat anti mouse to the cells in the absence of BrdU. The value from this control may vary with different cell lines and with high cell concentrations (greater than 2×10^4 cells/well).

Table 1. Summary of controls

Well contents	Assay blank	Non-specific binding control
Culture medium	100 µl	-
Cells	-	100 µl
BrdU	100 µl	-
Nuclease/anti-BrdU	50 µl	50 µl
Cy5 labelled goat anti mouse IgG	50 µl	50 µl
Hoechst (optional)	100 µl	100 µl

2. Cell culture

2.1. Culture cells (~10^3 – 10^4 cells/well are sufficient for most experiments depending on the cell type and the duration of the experiment) together with various dilutions of test substance (eg. mitogens, growth factors, cytokines, cytostatic drugs) in a 96-well microplate (tissue culture grade, flat bottom) in a final volume of 100 µl/well in a humidified atmosphere at 37°C in 5% CO_2.

2.2. The incubation period for cell culture depends on the particular experimental approach and on the cell type used for the assay. For most experiments an incubation time of 18 to 120 hours is appropriate. Cells should be passaged and cultured under the conditions routinely employed for the cell line being used for the assay. Where possible, avoid the use of media containing thymidine. Media may be used with or without serum supplementation, as appropriate to the experiment.

3. Labelling the cells with BrdU

3.1. Add 100 µl/well previously diluted BrdU labelling reagent (refer to Reagent Preparation section) directly to cultured cells (providing the cells are cultured in 100 µl/well volumes).

If cells are cultured in a different volume, adjust the concentration and volume of the BrdU labelling solution accordingly to ensure that the BrdU is at a final dilution of 1:500 in the well.

3.2. Reincubate cell cultures for an additional 1 to 24 hours at 37°C in 5% CO_2. For most applications, a 3 hour labelling time is adequate.

Extension of the incubation time will increase the amount of BrdU incorporated into cellular DNA and thus will increase the fluorescent signal obtained, and the assay sensitivity. This may be important for detection on some instruments.

4. Removal of labelling medium

For adherent cells

Remove labelling medium by decanting or aspiration.
Wash cells with 200 µl sterile PBS/well.

5. Cell fixation

5.1. Add 200 µl/well fixing solution (refer to Reagent Preparation section) to all wells and incubate for 18–24 hours at +4 °C.

5.2 Remove fixing solution by decanting or aspiration. Wash cells three times with 200 µl PBS/well.

6. Incubation with anti-BrdU/Nuclease reagent

6.1. Add 50 µl/well anti-BrdU/Nuclease reagent (refer to Reagent Preparation section).

6.2. Incubate for 45 min at 37°C. This incubation period can be varied between 30–60 min to obtain an optimal signal, however this should be optimized for each cell line.

Note: if this incubation is left for too long, the assay signal may decrease with time as excess nuclease treatment may cause loss of incorporated BrdU from DNA.

6.3. Remove anti-BrdU/Nuclease reagent by decanting or aspiration. Wash cells three times with 200 µl PBS/well.

7. Incubation with Cy 5 labelled goat anti mouse IgG

7.1. Add 50 µl/well Cy 5 labelled goat anti mouse reagent at a concentration of 10 µg/ml (refer to Reagent Preparation section).

7.2. Incubate for 1 hour at Room Temperature in the dark. Alternatively, the plate may be wrapped in aluminium foil.

7.3. Remove Cy 5 labelled goat anti mouse reagent by decanting or aspiration. Wash cells three times with 200 µl PBS/well.

7.4. Leave the cells in 100 µl PBS/well.

Note: the plate may be wrapped in aluminium foil and stored for up to 1 week at +4ºC prior to analysis.

8. Nuclear staining for microscopy. OPTIONAL.

Staining of nuclei is required to facilitate image analysis on some automated sub-cellular imagers.

8.1. Decant PBS from all wells and add 100 µl/well 2 µM Hoechst nuclear dye (e.g., Molecular Probes 33342).

8.2. Incubate for 30 min at Room Temperature in the dark.

8.3. Remove Hoechst nuclear dye by decanting or aspiration. Wash cells three times with 200 µl PBS/well.

8.4. Leave the cells in 100 µl PBS/well.

Fig 1. Flow chart of assay procedure

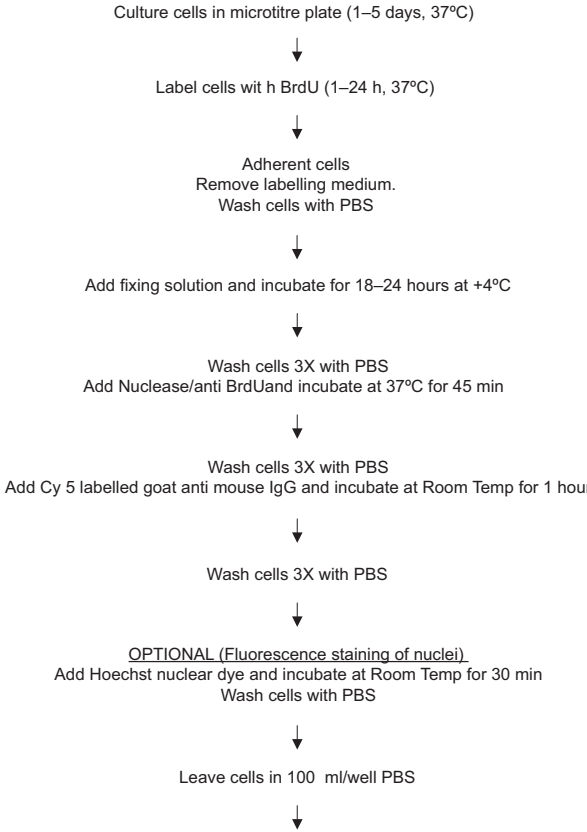

Image acquisition and analysis using the IN Cell Analyzer 1000 and IN Cell Analyzer 3000

Image Acquisition using the IN Cell Analyzer 1000:
The following filter sets are used for image acquisition:
excitation 360 nm and 620 nm, emission 535 nm and 700 nm

Dichroic: D / Cy 5

Objective: a X10 objective is sufficient for this assay

For more information please refer to the IN Cell Analyzer 1000 User's Guide.

Image Acquisition using the IN Cell Analyzer 3000:
The following filter sets are used for image acquisition:
Cy 5, excitation 647 nm, emission 695–55 nm
Hoechst, excitation 364 nm, emission 450–25 nm

Objective: X10 ELWD Nikon. 0.6 NA.

For more information please refer to the IN Cell Analyzer 3000 User's Guide.

Image Analysis using the IN Cell Analyzer 1000 and IN Cell Analyzer 3000:
Analyze the images from IN Cell Analyzer 1000 or IN Cell Analyzer 3000 using the Object Intensity Analysis Module to quantitatively measure the nuclear fluorescence intensity per cell.

For more information please refer to the IN Cell Analyzer 1000 or IN Cell Analyzer 3000 Object Intensity Analysis Module User's Guide.

7.3 Data Processing

Calculation of results (Plate reader).

Fluorescence intensity of BrdU labelled cells:

RFU BrdU labelled cells – RFU Non-specific binding control where:

RFU = relative fluorescence units

Signal to Noise ratio:

To calculate the Signal: Noise ratio, it is recommended that the following formula is used:

$$\frac{\text{(Mean RFU BrdU labelle cells) - (Mean RFU Non-specific binding control)}}{\sqrt{\text{(SD RFU BrdU labelle cells)}^2 + \text{(SD RFU Non-specific binding control)}^2}}$$

where:

RFU = relative fluorescence units

SD = standard deviation

8. Troubleshooting guide

As cell culture systems vary in cell number, proliferating activity and require different incubation periods, most problems will occur because the fluorescent signal is either too high or low. Assay conditions may be altered in order to achieve the optimum signal.

Procedures for optimization may differ according to the instrumentation and method used to increase the assay signal, e.g., instrumentation such as plate readers which do not distinguish background fluorescence from specific nuclear fluorescence may require extensive optimization.

Problem	Possible causes
1. Fluorescent signal too low	**1.1.** Increase labelling time with BrdU (1–24 h). **1.2.** Check cells were not lost during assay procedure. **1.3.** Increase cell number or incubation period. **1.4.** Check dilution of BrdU labelling reagent used. **1.5.** Re-optimize incubation time with Nuclease/anti BrdU reagent (this may require increasing or decreasing, depending upon the cell type). **1.6.** Ensure reagents are used within 4 weeks of preparation. **1.7.** Check medium formulation for presence of thymidine.

Possible causes	Possible causes
2. Fluorescent signal too high	**2.1.** Decrease cell number or incubation period. **2.2.** Decrease labelling time with BrdU to 1 hour. **2.3.** Decrease concentration of Cy 5 labelled goat anti mouse IgG.
3. High background control	**3.1.** Ensure that cells are washed 3X with PBS following incubation with anti BrdU primary antibody and Cy 5 labelled goat anti mouse IgG. **3.2.** Decrease concentration of Cy 5 labelled goat anti mouse IgG. **3.3.** Some cells may show an increase in non-specific binding at high cell concentrations (above 2 x 10^4 cells/well). Reducing the cell concentration should overcome this problem.

9. Additional Information

9.1 Specificity

The specificity of the antibody has been tested by competitive ELISA; binding was inhibited by 5-Bromo-2'-deoxyuridine, 5-Chloro-2'-deoxyuridine and 5-Iodo-2'-deoxyuridine. No cross-reactivity was observed with 5-Fluoro-2'-deoxyuridine or Thymidine.

Sensitivity

Depending on the individual cell type and the incubation time used for the assay, 10^3–10^4 cells/well are sufficient for most experiments.

9.2 Sensitivity

Depending on the individual cell type and the incubation time used for the assay, 10^3–10^4 cells/well are sufficient for most experiments.

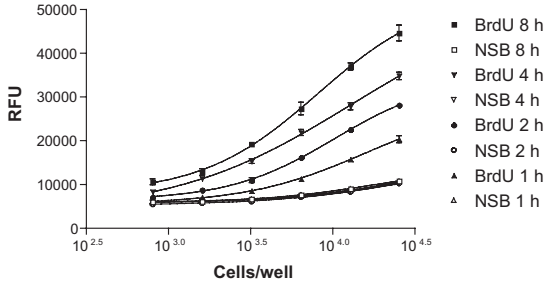

Fig 2. Sensitivity and kinetics of the cell proliferation assay.

U2-OS (human osteosarcoma) cells were incubated for 18 h in micro plates. After 18 h incubation, BrdU was added and the cells reincubated for an additional 1 h (▲), 2 h (●), 4 h (▼) or 8 h (■). BrdU incorporation was determined as described in the assay procedures section. The relative fluorescence intensity (RFU) was measured using

a Tecan Ultra fluorescence plate reader

Mean +/- SEM, n = 5.

BrdU = Cells labelled with BrdU

NSB = Non-specific binding control.

Note: Increasing the labelling time with BrdU increased the relative fluorescence intensity (RFU). Higher cell concentrations significantly increased the RFU, with a small increase in NSB values.

9.3 Reproducibility

Intra-assay precision

To determine the intra-assay variance, three assays were performed, each by a different operator, using replicates of ten in each assay. A variance of <8% was established for the relative fluorescence intensity (RFU) values when measured using a Tecan Ultra fluorescence plate reader. U2OS cells were seeded at 7 000 cells/well and labelled with BrdU for 3 hours. BrdU incorporation was determined as described in the assay procedures section.

	Average RFU	SD	%CV	n	BrdU labelled cells with Non-specific binding control subtracted
BrdU labelled cells	44186.8	2265.9	5.1	30	29162.1
NSB (Non-specific binding control)	15024.8	1135.2	7.6	30	

Inter-assay precision

To determine the inter-assay variance, the assay was performed three times over three successive weeks. Each assay was set up with replicates of ten. A variance of <8% was established for the relative fluorescence intensity (RFU) values when measured using a Tecan Ultra fluorescence plate reader. Cells were seeded at 7 000 cells/well and labelled with BrdU for 3 hours. BrdU incorporation was determined as described in the assay procedures section.

	Average RFU	SD	%CV	n	BrdU labelled cells with Non-specific binding control subtracted
BrdU labelled cells	42809.5	2886	6.7	3	27828.8
NSB (Non-specific binding control)	14980.7	1013.1	6.8	3	

9.4 Sample data

Inhibition data – plate reader

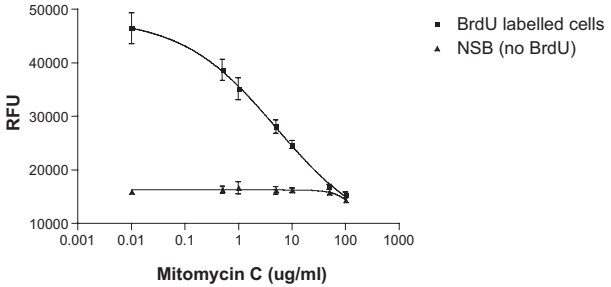

Fig 3. Dose response showing BrdU labelled cells and Non-specific binding control following treatment with Mitomycin C.

U2-OS (human osteosarcoma) cells were seeded at 7 000 cells/well and incubated with Mitomycin C for 7 h in microtitre plates. After 4 h incubation, BrdU was added and the cells reincubated for an additional 3 h. BrdU incorporation was determined as described in the assay procedures section. The relative fluorescence intensity (RFU) was measured using a Tecan Ultra fluorescence plate reader. Each data point represents the mean +/- SD of six replicates.

Inhibition data – imaging platform

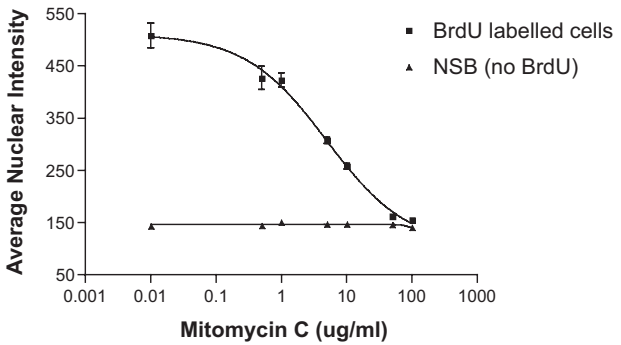

Fig 4. Dose response showing BrdU labelled cells and Non-specific binding control following treatment with Mitomycin C.

The average fluorescence nuclear intensity/well was measured using an IN Cell Analyzer 1000 and the data analysed using the Object Intensity algorithm (GE Healthcare). Refer to figure 3 for assay conditions. Each data point represents the mean +/- SD of six replicates.

Imaging data

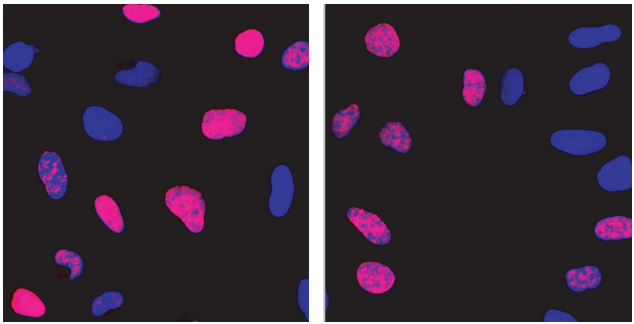

Fig 5. Nuclei of U2OS cells showing BrdU incorporation into DNA. (Images from same well, different fields of view).

U2-OS (human osteosarcoma) cells were seeded at 7 000 cells/well and incubated for 18 h in micro plates. BrdU was then added and the cells reincubated for an additional 1 h. BrdU incorporation was determined as described in the assay procedures section. Nuclei were stained with Hoechst nuclear dye (blue). BrdU incorporation is shown in pink (Cy 5). Images were acquired using an IN Cell Analyzer 1000 (GE Healthcare).

Imaging data - Multiplexing

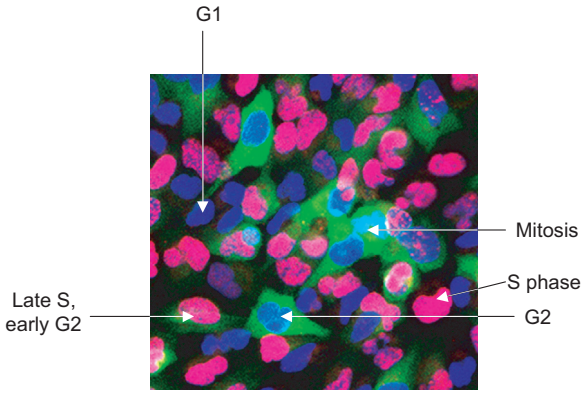

Fig 6. Multiplexing the fluorescence cell proliferation assay with GE Healthcares' G2M cell cycle phase marker cell line (10).

GE Healthcares' G2M cell cycle phase marker cell line stably expresses a GFP fusion protein which follows the expression and degradation kinetics of Cyclin B1. When multiplexed with the fluorescence cell proliferation assay, cells at all four stages of the cell cycle can be identified.

Cells were seeded at 7 000 cells/well and incubated for 18 h in micro plates. BrdU was then added and the cells reincubated for an additional 1 h. BrdU incorporation was determined as described in the assay procedures section. Nuclei were stained with Hoechst nuclear dye (blue). BrdU incorporation is shown in pink (Cy 5). Images were acquired using an IN Cell Analyzer 1000 (GE Healthcare).

10. Background

Traditionally, cell proliferation *in vitro* is determined by counting cells directly, by the determination of the mitotic index or, in the case of haematopoietic cells, by performing a clonogenic assay. All these assays are labour-intensive and are not practical for evaluating large numbers of samples. Alternatively, as an indirect measure of viable cell number, the overall metabolic activity in a cell population may be determined. Tetrazolium salts like MTT, XTT or WST-1 are metabolized by NAD-dependent dehydrogenase activity to form a coloured reaction product. In these assays the amount of dye formed directly correlates to the number of viable cells. These assays are performed in 96-well micro plates and the results are easily quantified with a standard ELISA reader, allowing the processing of large sample numbers. However such assays, which measure the number of metabolically active cells, would fail when, for example, a small number of proliferating cells are masked by an overwhelming majority of non-proliferating cells (e.g., antigen-specific stimulation of lymphocytes); or when DNA synthesis is induced in an arrested cell population without any change in cell number or cell viability (e.g. short-term measurement of growth factor activity on 3T3 or AKR-2B cells).

Since cellular proliferation requires the replication of cellular DNA, the monitoring of DNA synthesis is another indirect parameter of cell proliferation as well as being suitable for the study of the regulation of DNA synthesis itself. DNA synthesis has been the most common measure of mitosis and cell proliferation, and [3H]-thymidine has traditionally been used to label the DNA of mitotically active cells. Disadvantages of the [3H]-thymidine incorporation assay are: (1) handling and disposal problems of radioisotopes; (2) the requirement of specialized and expensive equipment like a cell harvester and scintillation counter, and (3) the hazards associated with the

handling of scintillation fluids. These disadvantages have led to the development of non-radioactive replacements for this assay.

An important improvement has been the replacement of [3H]-thymidine by 5-bromo-2'-deoxyuridine (BrdU). This technique is based on the incorporation of the pyrimidine analogue, BrdU instead of thymidine into the DNA of proliferating cells. After its incorporation into DNA, BrdU is detected by immunoassay. Several monoclonal antibodies which are highly specific for BrdU have been described (1–6). Nuclease digestion of DNA enables antibody access to the incorporated BrdU without adversely affecting cell/tissue morphology (7–8). Original techniques involved immunohistochemical detection of cells during the S-growth phase and, quantification of cell proliferation by microscopic or flow cytometric analyses. Although very informative, these techniques do not allow a high sample throughput in routine cell proliferation studies.

The fluorescence cell proliferation assay is designed as a precise, fast and simple fluorescence alternative to quantitate cell proliferation based on the measurement of BrdU incorporation during DNA synthesis of proliferating cells. The fluorescence cell proliferation assay can be used in many different *in vitro* cell systems when cell proliferation has to be determined.

The use of a Cy 5 red-shifted fluor enables this technology to be multiplexed with GFP. It can be used in conjunction with other GFP cell based assay technologies to detect compounds with unwanted toxicity. This assay also provides a high-throughput method of determining cell cycle position of cell populations which is currently performed by FACS and is not amenable to high-throughput.

Examples:

- Detection and quantification of cell proliferation induced by growth factors and cytokines.
- Determination of inhibitory or stimulatory effects of various compounds on cell proliferation in environmental and biomedical research and in the food, cosmetic and pharmaceutical industries.
- Determination of the immunoreactivity of lymphocytes, stimulated by mitogens or antigens.
- Determination of the chemosensitivity of tumour cells to different cytostatic drugs in medical research.
- Determination of the proportion of cells in S phase of the cell cycle.
- Identification of cells in S phase of the cell cycle.
- Used to identify the cell cycle position of all cells in asynchronous populations in conjunction with GFP G2M cell cycle phase marker (GE Healthcare).

11. References

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12. Related products

Cell Proliferation Kit	RPN 20
Cell Proliferation Biotrak ELISA System	RPN 250
Cy5 labelled goat anti-mouse IgG	PA45002
Cell Proliferation Labelling Reagent	RPN 201
Anti Bromodeoxyuridine + Nuclease	RPN 202
G2M phase marker screening	25-8010-50
G2M phase marker research	25-8010-51
G2M phase marker 6MTH AEL	25-8010-52
G2M phase marker 12 MTH AEL	25-8010-53
IN Cell Analyzer 3000	25-8010-11
IN Cell Analyzer 1000	25-8010-26
Object Intensity analysis module (for IN Cell Analyzer 1000)	25-8010-56
Object Intensity analysis module (for IN Cell Analyzer 3000)	63-0048-93
Cell Cycle Trafficking analysis module (for IN Cell Analyzer 3000)	63-0050-71

Please contact your local GE Healthcare office for details.

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