VWR® Automated Cell Counter Fluo – Viability/Cytotoxicity Assay Kit

Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells

Assay Principle:

Calcein AM is membrane permeant and can be introduced into cells via incubation. Once inside the cells, non-fluorescent Calcein AM is hydrolyzed by cellular esterases into the green-fluorescent dye Calcein. Calcein dye is water soluble and highly negatively charged and is only retained in the cytoplasm of healthy cells.

Ethidium Homodimer III (EthDIII) is a membrane-impermeant DNA/RNA binding dye that selectively stains dead cells with damaged membranes with red fluorescence. It has higher affinity and brightness compared to the commonly used dead cell dye Propidium Iodide.

Materials:

- VWR® automated cell counter Fluo. Cat. No. 49893-2000
- VWR® cell counting slide (2 samples/slide). Cat. No. 10228-0050
- VWR® Fluo cube for GFP and AO, green. Cat. No. 49893-4951
- VWR® Fluo cube for PI, red. Cat. No. 49893-4952
- Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells:
    - 150 Assays: Cat. No. EU BTIU30002-T ; Cat. No. NA 89260-208
    - 300 Assays: Cat. No. EU BTIU30002 ; Cat. No. NA 89138-886
  - Kit Components:
    - Calcein AM 4 mM in anhydrous DMSO
    - EthD-III 2 mM in DMSO/H2O

Procedure:

1. Centrifuge the cell sample at 350 xg for 3 minutes to pellet the cells.
2. Remove the culture medium, taking care not to disturb the cell pellet. Resuspend the cell pellet in PBS (Cat. No. EU 392-0442) by gently pipetting up and down.

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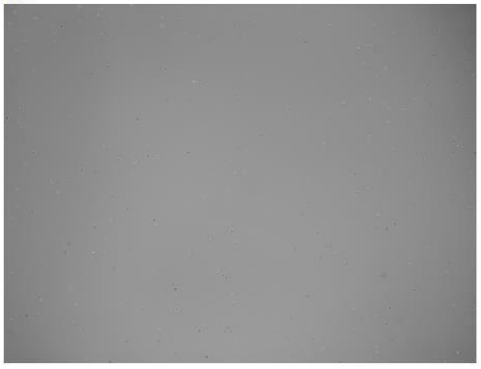
3. Prepare 2X staining solution just before use by mixing the following in a separate clean tube:
   1 uL of 4 mM Calcein AM
   4 uL of 2 mM EthDIII
   1 mL of PBS

4. In a clean tube, combine 20 uL of cell sample from step 2 with 20 uL of 2X staining solution from step 3. Pipette up and down gently to mix. The final concentrations will be 2 uM Calcein AM and 4 uM EthD-III.
5. Incubate the sample in a 37°C incubator for 15-30 minutes.
   Note: Longer incubation times may be used; incubation time may require optimization for different cell lines.
6. Mix the cells again by gently pipetting up and down, and load 10 uL of the stained cells into the counting slide for analysis in the BF, AO, and PI channels.

BF



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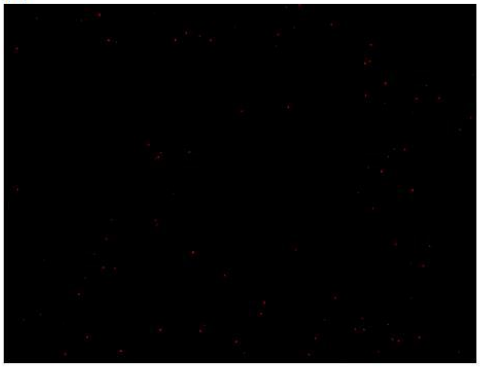
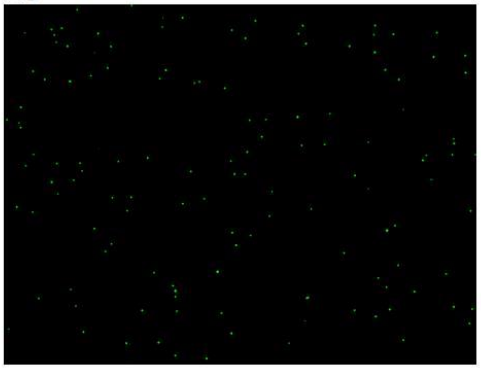


AO



BF+PI+AO