VWR® Automated Cell Counter Fluo – Calcein AM/PI Staining

Calcein AM and Propidium Iodide Live & Dead Co-Staining

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.

Propidium Iodide (PI) is a membrane-impermeant DNA/RNA binding dye that selectively stains dead cells with damaged membranes with red fluorescence.

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
- ☐ Calcein AM, 4 mMin DMSO. EU Cat. No. BTIU80011-1 ; NA Cat. No. 89139-470
- ☐ Propidium Iodide, 50 ug/mL in Buffer. EU Cat. No. BTIU40048 ; NA Cat. No. 89411-112

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 by gently pipetting up and down.
3. In a separate clean tube, prepare 2X Calcein AM/Propidium Iodide staining solution just before use as follows:
    a. Prepare an intermediate dilution of 100 uM Calcein AM by mixing 1 uL of 4 mM Calcein AM with 39 uL of PBS. Vortex to mix well.
    b. Add 1 uL of 100 uM Calcein AM to 24 uL of 50 ug/mL Propidium Iodide in Buffer. Vortex to mix well. Note: Scale volumes as needed for multiple samples.
4. In a clean tube, combine 20 uL of cell sample from step 2 with 20 uL of 2X staining solution from step 3b. Pipette up and down gently to mix. The final concentration of Calcein AM will be 2 uM and the final concentration of Propidium Iodide will be 25 ug/mL.

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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.