VWR® Automated Cell Counter Fluo – Calcein AM Staining

Calcein AM Live Cell Staining

Assay Principal:

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.

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
- Calcein AM, 4 mM in DMSO. EU Cat. No. BTIU80011-1 ; NA Cat.No. 89139-470

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 staining solution just before use. Combine 1 uL of 4 mM calcein AM and 1 mL of PBS. Vortex to mix well.
4. In a clean tube, combine 20 uL of cell sample from step 2 with 20 uL of 2X Calcein AM staining solution from step 3. Pipette up and down gently to mix. The final concentration of Calcein AM will be 2 uM.
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 and AO channels (deselect the PI channel).
7. Note: Calcein AM may be combined with Propidium Iodide (PI) for live/dead co-staining. We recommend using Calcein AM at a final concentration of 2 uM with PI at a final concentration of 25 ug/mL.

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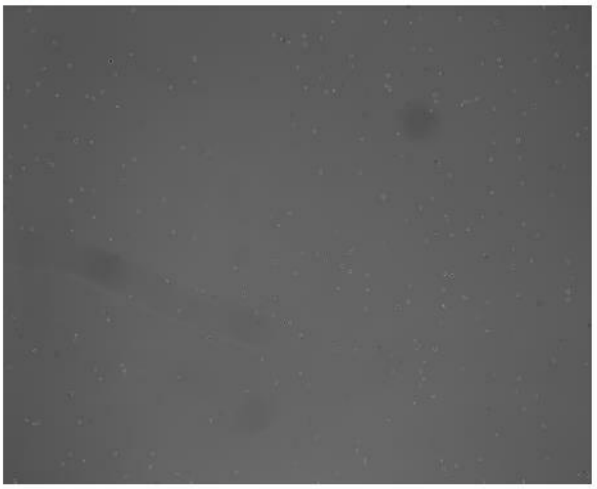
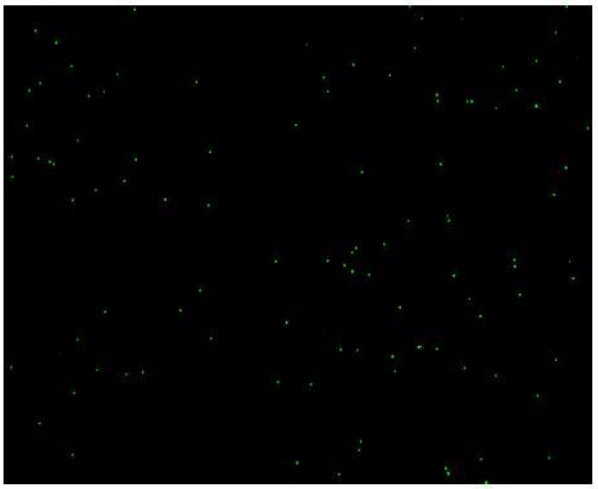
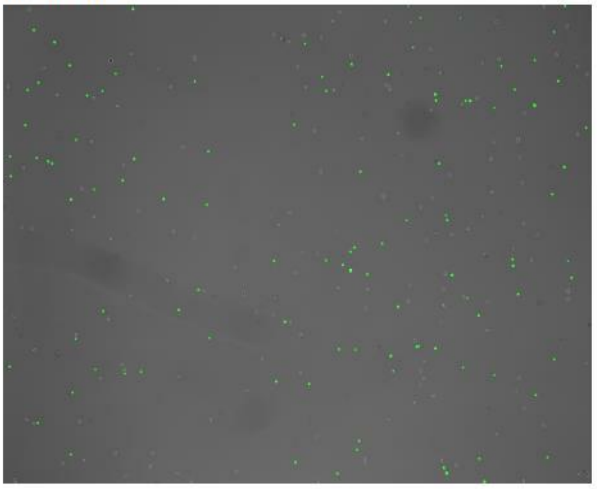
BF



AO



BF+AO



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