VWR® Automated Cell Counter FLUO –Hoechst staining

Hoechst Staining (Total Cell Stain)

Assay Principle:

Hoechst is a cell permeant DNA binding dye that stains the nuclei of both live cells and fixed cells with blue fluorescence. Note that dead cells may stain more brightly than live cells with Hoechst.

Materials:

- Hoechst 33342, 10 mg/mL in Water. Cat. No. 89139-124

Procedure:

Note: Cells may be stained in culture medium without washing.

1. Prepare 2X staining solution (20 ug/mL Hoechst in PBS) by adding 1 uL of 10 mg/mL Hoechst to 500 uL of PBS. Vortex to mix well.

   Note: Do not store the 2X staining solution. Use it within 2-3 hours of dilution.

2. Mix 20 uL of cells with 20 uL of 2X staining solution from step 1. Pipette up and down gently to mix.
3. Incubate 10 minutes at RT.
4. Mix the cells again by gently pipetting up and down, and then load 10 uL to the counting slide for analysis using the DAPI filter.

   Note: For rapid Hoechst staining in samples where no cell viability dye will be used, you may mix 10 uL of cells with 10 uL of 75% ethanol in water to kill and permeabilize the cells. Then add 20 uL of 2X staining solution to the cell/ethanol mixture. Pipette up and down to mix, then load 10 uL to the counting slide for imaging.