TONBO biosciences

Equivalent Performance, Exceptional Value

Ghost Dyes™ Staining Protocol

Ghost Dye™ viability dye (see http://www.tonbobio.com/ghost-dyes for available formats)

Other Materials Required

- 1X PBS (azide-free, protein/serum-free)
- Flow Cytometry Staining Buffer (Stain Buffer) (1X PBS with 2% FBS, 0.09% Na-Azide)

1. Remove Ghost Dye vial from freezer and allow to equilibrate to room temperature.
2. Quick spin Ghost Dye vial before opening.
3. Prepare azide- and protein/serum-free 1X PBS for labeling procedure.
4. Wash cells twice in 1-2 mL azide- and protein/serum-free 1X PBS. Spin at 300-400 x *g* for 5 minutes at room temperature and decant supernatant.
5. Resuspend cells to a concentration of 1-10 x 10^6/mL in azide- and protein/serum-free 1X PBS.
6. Add 1 uL of Ghost Dye solution for each 1 mL of cell suspension and vortex immediately. *Note*: Ghost Dyes are formulated in DMSO, pipet carefully and slowly.
7. Incubate for 30 minutes at 2-8°C protected from light.
8. Wash cells 1-2 times with 1-2 mL Stain Buffer. Washing with a protein containing buffer allows removal of any unreacted dye prior to staining with fluorescent antibodies.
9. Cells can be subsequently stained, fixed and permeabilized according to user protocol.

    Note: Cells labeled with Ghost Dyes can be cryopreserved for later use or used in intracellular staining protocols without any loss of fluorescence intensity.