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Directions for Use
Cytus™ Blue Cell Nucleus Stain

Cat# 5469-100UL

Product Overview

Cytus™ Blue is a ready-to-use nuclear staining solution designed for rapid and reliable visualization of cell nuclei in most mammalian cell types. The formulation is based on Hoechst 33342, a widely used bis-benzimide fluorescent dye that binds to the minor groove of DNA and produces bright blue/cyan fluorescence. With an excitation maximum near 350 nm and emission at 454 nm, Cytus™ Blue delivers high-contrast nuclear labeling ideal for fluorescence microscopy, imaging-based assays, and quantitative analysis.

Cytus™ Blue is lipophilic and can readily penetrate intact cell membranes, making it suitable for staining both live and fixed cells. This versatility allows researchers to incorporate Cytus™ Blue into workflows ranging from live-cell imaging to endpoint assays. The stain is compatible with fluorescence microscopy, micro-plate readers, cuvette-based measurements, and flow cytometry, providing flexibility across a variety of experimental platforms.

Characterization and Testing

Parameter/Tests	Specification
Concentration	1 mM in water
Volume	100 µL
No. of use	200 to 1000
Cell staining	Characteristic
Storage Temperature	-20 °C

Sample Staining Procedure

For adherent cells:

1. Culture cells according to the manufacturer's protocol in the tissue culture plates.

2. Remove cell culture medium and wash/rinse the cell monolayer with sterile 1X PBS twice. If staining live cells, skip to Step 4.

3. **Fixation:** Fix cells with 4% paraformaldehyde (10 to 30 min incubation at room temperature). Remove fixative and rinse cells with 1X PBS twice.

4. Dilute Cytus™ Blue stock to working concentrations between 1 and 5 µM using sterile DI water or 1X PBS.

5. **Staining**: add sufficient Cytus™ Blue working solution to cover the wells and incubate the plates protected from direct light at room temperature for 5 to 10 minutes.

Well Plate Type	Volume of Solution per Well
6-Well	1 to 3 mL
12-Well	1 to 2 mL
24-Well	0.5 to 1 mL
48-Well	200 to 400 µL
96-Well	100 to 200 µL

6. Remove the Cytus™ Blue Staining Solution and rinse the stained wells thoroughly with 1X PBS twice.

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7. Cover the stained cells with sufficient 1X PBS and proceed for imaging.

8. **Imaging:** Image the stained plates using the DAPI fluorescence (Ex/Em: 352/454 nm) filter.

For suspended cells:

1. Culture cells according to the manufacturer's protocol in tissue culture flasks.

2. Remove cell culture medium and collect cells using Trypsin-EDTA. Transfer cell suspension into a centrifuge tube.

3. Centrifuge the cells to yield a pellet. Carefully remove the supernatant, add 1X PBS and resuspend the cells.

4. Centrifuge the cells again and carefully remove the supernatant.

5. Dilute Cytus™ Blue stock to working concentrations between 1 and 5 μM using sterile DI water or 1X PBS.

6. **Staining**: add sufficient Cytus™ Blue working solution to cover the cell pellet and resuspend the cells in the staining solution. Incubate the cells protected from direct light at room temperature for 5 to 10 minutes.

7. **Washing:** centrifuge to pellet the cells after staining, remove the supernatant, and wash stained cells with 1X PBS twice to completely remove unbound dye.

8. Load cells for analysis (i.e. flow cytometry) or resuspend the stained cells in fresh media and plate them for subculture.

Sample image

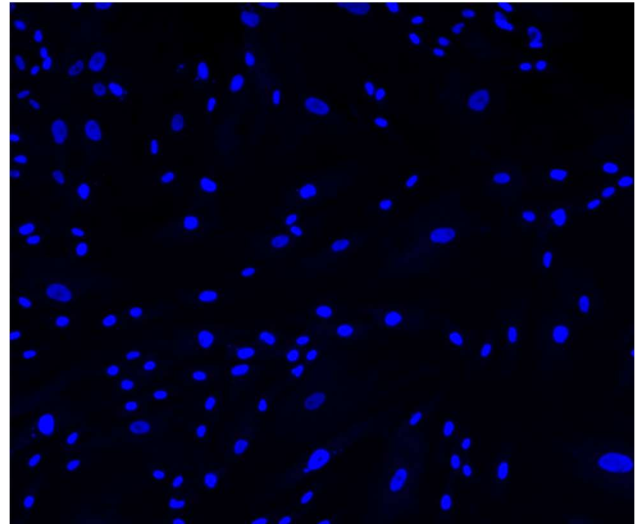

Fig.1. Human fibroblasts cultured in a CytoSoft™ 24-Well Imaging Plate. The cell nuclei were stained with Cytus™ Blue and exhibited blue fluorescence.

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