Cell Navigator™ Live Cell Endoplasmic Reticulum (ER) Staining Kit *Blue Fluorescence*

Catalog number: 22634
Unit size: 100 Tests

Component	Storage	Amount
Component A: ER Tracer™ Blue	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Live Cell Staining Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (20 mL)
Component C: DMSO	Freeze (< -15 °C)	1 vial (100 uL)

OVERVIEW

The endoplasmic reticulum (ER) is a type of organelle in the cells of eukaryotic organisms that forms an interconnected network of flattened, membrane-enclosed sacs or tube-like structures known as cisternae. The membranes of the ER are continuous with the outer nuclear membrane. ER occurs in most types of eukaryotic cells, but is absent from red blood cells and spermatozoa. This Cell Navigator™ Live Cell Endoplasmic Reticulum (ER) Staining Kit uses our ER Tracer™ Blue as an ER marker. ER Tracer™ Blue stain is a cell-permeant fluorescent dye that is highly selective for ER. This stain consists of a blue fluorescent dye and ER binder that selectively bind to ER in most of cell types. For some cells, ER Tracer™ Blue may not selectively bind to ER. ER Tracer™ Blue has spectral properties similar to DAPI, making this kit convenient with the DAPI filter set.

AT A GLANCE

Protocol Summary

1. Prepare cells in growth medium
2. Incubate cells with ER Tracer™ Blue working solution at 37° C for 15 - 30 minutes
3. Analyze under fluorescence microscope with DAPI filter set

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microscope

Excitation	DAPI filter set
Emission	DAPI filter set
Recommended plate	Black wall/clear bottom

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

ER Tracer™ Blue stock solution (500X)
Add 20 µL of DMSO (Component C) into ER Tracer™ Blue (Component A) to make 500X stock solution.

PREPARATION OF WORKING SOLUTION

ER Tracer™ Blue working solution
Add 2 µL of 500X stock solution into 1 mL of Live Cell Staining Buffer (Component B), and mix well. The working solution is stable for at least 2 hours at room temperature.

Note 20 µL of 500X ER Tracer™ Blue stock solutions is enough for one 96-well plate. Unused ER Tracer™ Blue 500X stock solution can be aliquoted and stored at ≤ -20 ° C for two weeks if the tubes are sealed tightly. Protect from light and avoid repeated freeze-thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

1. Treat samples as desired.

   Note Working solution can be added directly into the cell culture medium. Alternatively, remove the cell culture medium and wash with the buffer of your choice.

2. Add 100 µL/well (96-well plate) or 50 µL/well (384-well plate) of ER Tracer™ Blue working solution in the cell plate. Incubate cells with working solution at 37° C for 15-30 minutes, protected from light.

   Note The optimal concentration of the ER probe varies depending on the specific application. Concentration higher than the working solution can be toxic to cells. The staining conditions may be modified according to the particular cell type and the permeability of the cells or tissues to the probe.

3. Remove working solution in each well. Wash cells with physically relevant buffer three times.

4. Fix cells after staining (Optional). Fix the cells with 4% formaldehyde for 5 -10 minutes. Wash cells with physically relevant buffer three times.

5. Observe the fluorescence signal in cells using fluorescence microscope with a DAPI filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

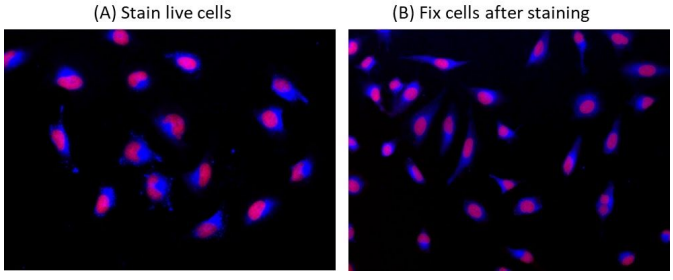

Figure 1. Fluorescence images of endoplasmic reticulum (ER) staining in HeLa cells cultured in a 96-well black-wall clear-bottom plate using fluorescence microscope with a DAPI filter set. (A) Live cells were co-stained with ER-selective probe ER Tracer™ Blue (Cat#22634, Blue) and Nuclear Red™ LCS1 (Cat#17542, Red). (B) Live cells were co-stained with ER-selective probe ER Tracer™ Blue (Cat#22634, Blue) and Nuclear Red™ LCS1 (Cat#17542, Red) and then were fixed with 4% formaldehyde.

DISCLAIMER

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