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

Catalog number: 22636
Unit size: 100 Tests

Component	Storage	Amount
Component A: ER Tracer™ Red	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 µL)

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™ Red as an ER marker. ER Tracer™ Red stain is a cell-permeant fluorescent dye that is highly selective for ER. This stain consists of a red fluorescent dye and ER binder that selectively bind to ER in most of cell types. For some cells, ER Tracer™ Red may not selectively bind to ER. ER Tracer™ Red has spectral properties essentially identical to Texas Red, making this kit convenient with the Texas Red filter set.

AT A GLANCE

Protocol Summary

1. Prepare cells in growth medium
2. Incubate cells with ER Tracer™ Red working solution at 37 °C for 15 - 30 minutes
3. Analyze under fluorescence microscope at Ex/Em = 590/620 nm (TRITC or Cy3 filter set)

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

KEY PARAMETERS

Fluorescence microscope

Excitation	590 nm
Emission	620 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	TRITC or Cy3 filter

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™ Red stock solution (500X)
Add 20 µL of DMSO (Component C) into the vial of ER Tracer™ Red (Component A) and mix well to make 500X ER Tracer™ Red stock solution.

Note 20 µL of 500X ER Tracer™ Red stock solution is enough for one 96-well plate. Unused 500X ER Tracer™ Red stock solution can be stored at ≤ -20 ºC for two weeks if the tubes are sealed tightly. Protect from light.

PREPARATION OF WORKING SOLUTION

ER Tracer™ Red working solution
Add 20 µL of 500X ER Tracer™ Red stock solution into 10 mL of Live Cell Staining Buffer (Component B), and mix well to make ER Tracer™ Red working solution.

Note This ER Tracer™ Red working solution is stable for at least 2 hours at room temperature. Protect from light.

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 100 µL/well (96-well plate) or 50 µL/well (384-well plate) of ER Tracer™ Red 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.

2. Remove ER Tracer™ Red working solution in each well. Wash cells with physically relevant buffer three times.

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

4. Observe the fluorescence signal in cells using fluorescence microscope with TRITC or Cy3 filter set (Ex/Em = 590/620 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

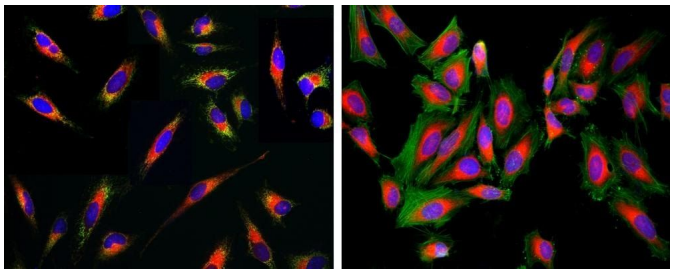

Figure 1. Fluorescence images of endoplasmic reticulum (ER) staining in HeLa cells cultured in a 96-well black-wall clear-bottom plate using fluorescence microscope with a TRITC filter set. Left: Live cells were stained with ER-selective probe ER Tracer™ Red (Cat#22636, Red), mitochondria dye MitoLite™ Green (Cat#22675, Green) and nuclei stain Hoechst 33342 (Cat#17530, Blue). Right: Live cells stained with ER Tracer™ Red (Cat#22636, Red) were fixed with 4% formaldehyde, then labeled with F-actin dye iFluor™ 488-Phalloidin (Cat#22661, Green) and nuclei stain DAPI (Cat#17507, Blue).

DISCLAIMER

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