Cell Navigator™ Live Cell Endoplasmic Reticulum Staining Kit *Green Fluorescence*

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22635 (100 assays)	Keep in freezer and protect from light	Fluorescence microscope

Introduction

All eukaryotic cells have an endoplasmic reticulum (ER). The endoplasmic reticulum (ER) is a network of membrane-enclosed tubules and cisternae that extends from the nuclear membrane throughout the cytoplasm. It plays a major role in the production of many transmembrane proteins and lipids for its membrane and for many other cell components including lysosomes, secretory vesicles and the Golgi apparatus. The ER also functions as the transportation system in eukaryotic cells, responsible for carrying various proteins specifically to the Golgi apparatus.

ER Green™ cell-permeant probe enable staining of the live cell endoplasmic reticulum (ER) across a wide variety of mammalian cell types. Our Cell Navigator™ Live Cell Endoplasmic Reticulum Staining Kit provides excellent and rapid staining with high selectivity for ER over other cellular compartments such as mitochondria. The fluorescence staining in live cell ER is also maintained after fixation with formaldehyde, enabling further multi-color staining. In addition, the kits can be adapted for many different types of fluorescence platforms, such as fluorescence microscope, microplate assays and flow cytometry.

Kit Components

Components	Amount
Component A: ER Green™	1 vial
Component B: Live Cell Staining Buffer	1 bottle (20 mL)
Component C: DMSO	1 vial (100 µL)

Assay Protocol

Brief Summary

Prepare cells in growth medium → Incubate cells with ER Green™ working solution → Analyze under fluorescence microscope at Ex/Em = 490/520 nm (FITC filter set)

1. Prepare cells:

1.1. For adherent cells: Plate cells overnight in growth medium at 10,000 to 40,000 cells/well/90 µL for a 96-well plate or 2,500 to 10,000 cells/well/20 µL for a 384-well plate.

1.2. For non-adherent cells: Centrifuge the cells from the culture medium and suspend the cell pellets in culture medium at 50,000-100,000 cells/well/90 µL for a 96-well poly-D lysine plate or 10,000-25,000 cells/well/20 µL for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to your experiment.
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

2. Prepare working solution:

2.1 Thaw kit components at room temperature before use.

2.2 Make ER Green™ 500X stock solution: Add 20 µL of DMSO (Component C) into ER Green™ (Component A) to make 500X stock solution.

2.3 Make ER Green™ working solution: Add 20 µL of 500X stock solution (Component A) into 10 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 Green™ stock solution (Component A) is enough for one 96-well plate. Unused ER Green™ 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.

3. Stain cells:

3.1 Add 100 µL/well (96-well plate) or 50 µL/well (384-well plate) of ER Green™ working solution (from Step 2.3) 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.

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

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

3.4 Observe the fluorescence signal in cells using fluorescence microscope with a F filter set.

Data Analysis

(A) Stain live cells **(B) Fix cells after staining**

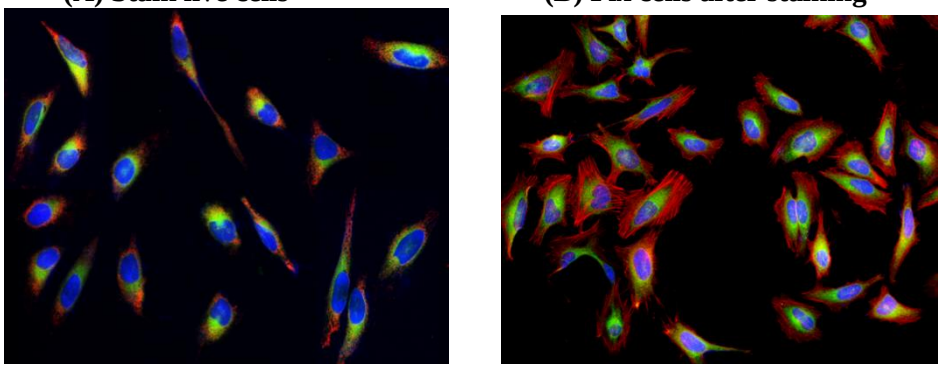

Figure 1. . Fluorescence images of endoplasmic reticulum (ER) staining in HeLa cells cultured in a 96-well black-wall clear-bottom plate using fluorescence microscope with a FITC filter set. (A) Live cells were stained with ER-selective probe ER Green™ (Cat#22635, Green), mitochondria dye MitoLite™ Red FX600 (Cat#22677, Red) and nuclei stain Hoechst 33342 (Cat#17530, Blue). (B) Live cells stained with ER Green™ (Cat22635, Green) were fixed with 4% formaldehyde, and labeled with F-actin dye iFluor™ 594-Phalloidin (Cat#23122, Red) and nuclei stain DAPI (Cat#17507, Blue).

References

1. Baumann O, Walz B. (2001) Endoplasmic reticulum of animal cells and its organization into structural and functional domains. International Review of Cytology. 205: 149-214
2. Sabnisa RW, Deligeorgievb TG, Jachakc MN and Dalvi TS. (1997) DiOC6(3): a useful dye for staining the endoplasmic reticulum. Biotechnic and Histochemistry. 72 (5): 253-258.
3. Samali A, FitzGerald U, Deegan S and Gupta1 S. (2009) Methods for monitoring endoplasmic reticulum stress and the unfolded protein response. International Journal of Cell Biology. 2010 (2010)