Cell Navigator™ Cell Plasma Membrane Staining Kit *Deep Red Fluorescence*

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22681 (500 assays)	Keep in freezer and protect from light	Fluorescence microscope

Introduction

The cell membrane (plasma membrane) is a thin semi-permeable membrane that separates the interior of all cells from the environment. The basic function of the cell membrane is to protect the cell from its surroundings. It is composed mainly of lipids and proteins. Cell membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity and cell signaling and serve as the attachment surface for several extracellular structures, including the cell wall, glycocalyx, and intracellular cytoskeleton.

The Cellpaint™ Deep Red cell membrane probe used in the kit enables the uniform staining of cell membrane across a wide variety of mammalian cell types. The Cell Navigator™ Cell Membrane Staining Kit provides an excellent tool for the rapid staining of plasma membranes in suspended or attached live cells depending on the cell type and experimental conditions. The fluorescence staining in cell membranes is also maintained after fixation with formaldehyde, enabling further multi-color staining. In addition, the kit provides robust and flexible staining in live and fixed cells, and can be adapted for many different types of fluorescence platforms, such as fluorescence imaging and flow cytometry.

Kit Components

Components	**Amount**
Component A: Cellpaint™ Deep Red	1 vial
Component B: Assay Buffer	1 bottle (50 mL)
Component C: DMSO	200 µL

Assay Protocol

Brief Summary

Prepare cells in growth medium → Incubate cells with Cellpaint™ Deep Red working solution → Analyze under fluorescence microscope at Ex/Em = 640/660 nm (Cy5 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 one of each kit component at room temperature before use.

2.2 Make Cellpaint™ Deep Red 500X stock solution: Add 100 µL of DMSO (**Component C**) into the vial of Cellpaint™ Deep Red (**Component A**) to make 500X stock solution.

2.3 Make Cellpaint™ Deep Red working solution: Add 20 µL of 500X stock solution (**Component A**) into 10 mL of Assay Buffer (**Component B**), and mix well. The working solution is stable for at least 8 hours at room temperature.
Note: 20 µL of 500X Cellpaint™ Deep Red 500X stock solution (Component A) is enough for one 96-well plate. Unused Cellpaint™ Deep Red 500X stock solution can be aliquoted and stored at ≤ -20 °C for one month 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 Cellpaint™ Deep Red working solution (from Step 2.3) in the cell plate. Incubate the cells at 37 °C for 10-20 minutes, protected from light.
Note: The optimal concentration of the cell membrane probe varies depending on the specific application. 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 physiological buffer (such as HHBS, PBS or buffer of your choice) for three times.

3.3 Fix cells after staining (Optional). Fix the cells with 4% formaldehyde for 15-30 minutes. Wash cells with physiological buffer for three times.

3.4 Observe the fluorescence signal in cells using fluorescence microscope with a Cy5 filter set.

Data Analysis

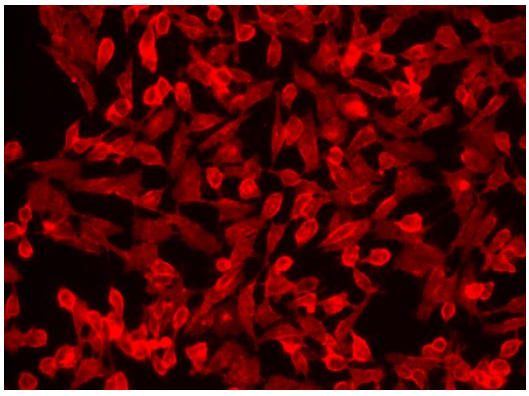

Figure 1. The fluorescence images of HeLa cells stained with Cellpaint™ Deep Red in a 96-well black-wall clear-bottom plate. The cells were imaged using fluorescence microscope with a Cy5 filter.

References

1. Brown DA and London E. (1998) Functions of lipid rafts in biological membranes. Annual Review of Cell and Developmental Biology. 14 (1): 111-136.
2. DePierre JW and Karnovsky ML. (1973) Plasma membranes of mammalian cells. Journal of Cell Biology. 56 (2): 275–303.
3. Besterman JM and Low RB. (1983) Endocytosis: a review of mechanisms and plasma membrane dynamics. Biochemical Journal. 210 (1): 1–13.