Version 1 Last updated 27 April 2017

ab219942 CytoPainter Cell Plasma Membrane Staining Kit – Deep Red Fluorescence

For staining cell plasma membrane in live cells using our proprietary deep red fluorescence probe

This product is for research use only and is not intended for diagnostic use.

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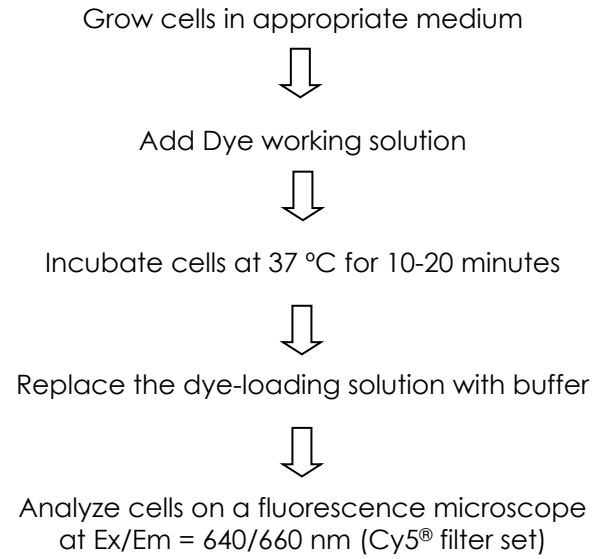
1. Overview

CytoPainter Cell Plasma Membrane Staining Kit – Deep Red Fluorescence (ab219942) uses a deep red cell membrane probe (Ex/Em = 640/660 nm) that enables the uniform staining of cell membrane across a wide variety of mammalian cell types. The 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.

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.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Deep Red Dye	1 vial	-20°C	-20°C
Assay Buffer	50 mL	-20°C	-20°C
DMSO	200 µL	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence microscope fitted with a filter capable of detecting fluorescence at Ex/Em = 640/660 nm (Cy5® filter set)
- HHBS (Hank's Balanced Salt solution + 20 mM HEPES buffer)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- General tissue culture supplies
- Sterile 96 well plate with clear flat bottom, preferably black (if performing assay in microplate format). Use a poly-D-lysine coated plate for suspension cells
- (Optional) 4% formaldehyde solution

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer (50 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 DMSO (200 µL):

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 min to thaw the DMSO solution before use.

Δ Note: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C. Repeat this step every time probe is needed.

Store at -20°C protected from light.

9.3 Deep Red dye (1 vial):

Reconstitute Deep Red Dye by adding 100 µL of DMSO to the vial to prepare **500X Deep Red Dye Stain Stock Solution**. Mix well by pipetting up and down.

Δ Note: 20 µL of Deep Red Dye Stain stock solution is enough for 1 x 96 well plate.

Aliquot unused stock solution so that you have enough volume to perform the desired number of assays. Store at -20°C with the cap sealed tightly away from light. Avoid repeated freeze-thaw cycles. Use within 2 months.

10. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.
- Each cell line should be evaluated on an individual basis to determine the optimal cell density.
- The protocol described here is for 96-well microplate format. You can adapt the protocol for other formats (384-well, 24-well, 6-well) by changing the volumes accordingly.

Δ Note: The optimal concentration and incubation time of the Deep Red Dye will vary depending on the specific application. The staining conditions may be modified according to the particular cell type and/or the permeability of the cells or tissues to the probe.

10.1 Grow cells:

10.1.1 Adherent cells: plate cells overnight in growth medium at 1-4 x 10^4 cells/90 µL per well.

Δ Note: for 384 well plate, use 2,5 x 10^3-10^4 cells/20 µL per well.

10.1.2 Suspension cells: on the day of the assay, centrifuge cells from the culture medium and resuspend the cell pellet in culture medium at 1-2 x 10^5 cells/90 µL per cell in a poly-D-lysine coated plate.

Δ Note: for 384 well plate, use 2.5-5 x 10^4 cells/20 µL per well.

10.1.3 Centrifuge plate at 800 rpm for 2 minutes with brake off.

10.2 Prepare Deep Red Cell Plasma Staining Solution:

10.2.1 Prepare dye working solution by diluting 20 µL of 500X Deep Red Dye Stock solution (Step 9.3) into 10 mL of Assay Buffer.

Δ Note: 20 µL of 500X Deep Red dye is enough for one 96 well plate. Do not store solution for longer than 8 hours.

10.3 Cell staining:

10.3.1 Add 100 µL/well of Deep Red Cell Plasma Staining Solution into the cell plate.

Δ Note: for 384 well plate, add 25 µL/well.

10.3.2 Incubate the cells for 10-20 minutes in a 37°C/5% CO_2 incubator, protected from light.

10.3.3 Aspirate dye-loading solution carefully.

10.3.4 Wash cells with HHBS or PBS (or another physiological buffer of your choice) 3 times.

10.3.5 Fix cells after staining (Optional step):

10.3.5.1 Fix cells with 4% formaldehyde solution for 15-30 minutes.

10.3.5.2 Wash cells with physiological buffer three times.

10.3.6 Observe the cells by using a fluorescence microscope fitted with a Cy5® filter set (Ex/Em = 640/660 nm).

11. Data Analysis

- We recommend acquiring several images per well.
- We recommend data analysis after coding and mixing images to ensure unbiased results.
- For manual analysis, if you do not have a specific software installed in your microscope, you can download ImageJ, an open source image processing designed for scientific multidimensional images by the National Institute of Health (NIH).

12. Typical Data

Data provided for **demonstration purposes** only.

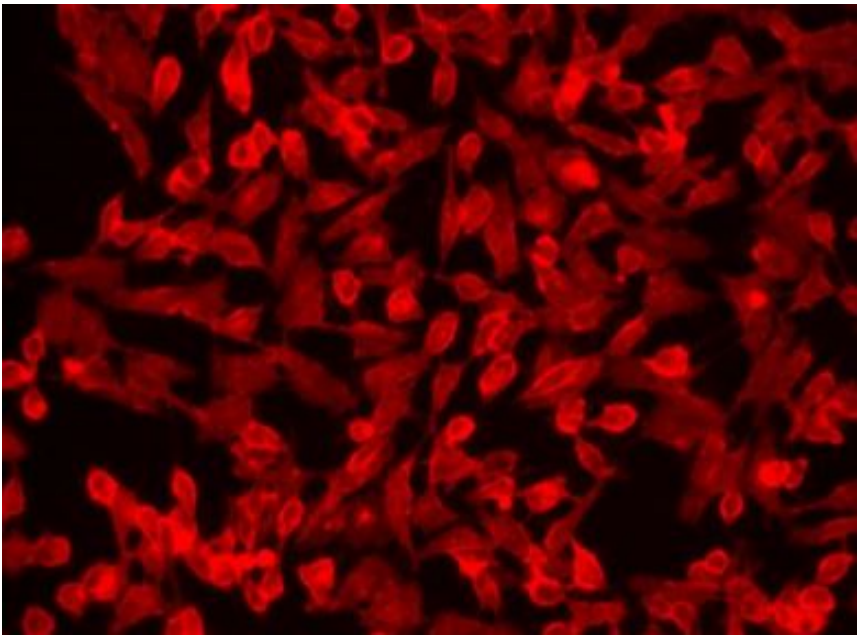


Figure 1. HeLa cells stained with CytoPainter Cell Plasma Membrane Staining Kit – Deep Red Fluorescence (ab219942) in a 96 well clear-bottom plate.

13. Notes

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