#A10020

细胞膜染色试剂盒
Cell Plasma Membrane Staining Kit (Green Fluorescence)

☐ 100T

Orders ■ 400-6123-828
orders@ab-mart.com
Web ■ www.ab-mart.com.cn

Background & Principle

The cell membrane (plasma membrane) is a thin semi-permeable membrane, consisting of a lipid bilayer with embedded proteins 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. The cell membrane controls the movement of substances in and out of cells and organelles. In this way, it is selectively permeable to ions and organic molecules. 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. #A10020 Cell Plasma Membrane Staining Kits are a set of fluorescence imaging tools for rapid staining of plasma membranes in living and fixed suspended or attached cells depending on the cell type and experimental conditions. The kit uses a proprietary lipophilic carbocyanine dye (Ex/Em = 484/501 nm) that weakly fluorescent in water but highly fluorescent and quite photostable when incorporated into membranes.

Storage/Stability

Refer to list of materials supplied for storage conditions of individual components. Stable for at least 6 months at recommended temperature from date of shipment. Gel pack with blue ice.

Assay Restrictions

• Assay kit is 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.

Materials supplied and Storage conditions

Kit components	Quantity	Storage conditions
PM Green (1000×)	50 uL	-20°C, Protect from light
Assay Buffer (10×)	5 mL	4°C

Other supplies required, Not Supplied

1. Microcentrifuge; 2. Pipettes and pipette tips; 3. Fluorescence Microscopy or Flow Cytometer;
4. 24-well plate for cell culture; 5. Phosphate-buffered saline (PBS);

Technical hints

1. To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
2. Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
3. Make sure all necessary equipment is switched on and set at the appropriate temperature.

ASSAY PROTOCOL

Reagent Preparation

PM Green (1000×): Warm to room temperature. Aliquot and store unused PM Green (1000×) stock solutions at -20°C. Protect from light and avoid repeated freeze-thaw cycles.
Assay Buffer: Prepare 1×Assay Buffer by dilute 10× Assay Buffer with ddH2O. Warm to 37°C before use.

#A10020

细胞膜染色试剂盒
Cell Plasma Membrane Staining Kit (Green Fluorescence)

☐ 100T

Orders ■ 400-6123-828
orders@ab-mart.com
Web ■ www.ab-mart.com.cn

Staining Solution: Mix 1 µl of PM Green (1000×) in each 1 ml of Assay Buffer. Scale up accordingly for larger numbers of assays.
Recommended procedures
Note: The optimal concentration of the PM Green and incubation time varies depending on the specific application. The staining conditions may need modified according to the particular cell type.

A. Quantification by Flow Cytometry

1. Treat cells with the desired method.
Note: We recommend keeping unstained control cells (i.e. without PM Green) suspended in Assay Buffer for both treated and untreated samples to set up the flow cytometer instrument.
2. For non-adherent cells, Collect 1-5 ×105 cells by centrifugation (4oC, 300g, 5min). Wash with ice-cold PBS twice and discard the PBS. For adherent cells, using Trypsin (EDTA free) to digest cells firstly and then centrifugation.
3. Resuspend the cells pellet in 500uL Staining Solution.
4. Incubate the cells at 37°C for 5-20 minutes in the dark.
5. Centrifuge cells at 500g and discard supernatant.
6. Wash cell pellet with PBS and repeat step 5.
7. Resuspend cell pellet in 0.5 ml of the pre-warmed PBS and analyze the cells by flow cytometry using FITC channel (usually FL1).

B. Detection by Fluorescence Microscopy

1. For suspension cells: Follow the protocol for flow cytometry from step1 to step4 and place the cell suspension from Step A.4 on a glass slide. Cover the cells with a glass coverslip. Analyze cells by fluorescence microscopy using the appropriate filters as soon as possible.
2. For adherent cells: the suggested protocol is as below.
2.1. Grow cells directly on a coverslip in 24 well dish. Incubate in a CO2 Incubator at 37°C for at least 24 hours before treatment.
2.2. Wash cells with PBS twice.
2.3. Add 0.5 mL of Staining solution to cells and incubate at 37°C for 5-10 minutes in the dark.
2.4. Wash cells with pre-warm PBS twice.
2.5. Fix cells after staining (Optional): Fix the cells with 4% paraformaldehyde for 15-30 minutes. Other fixatives, particularly glutaraldehyde, tend to produce unacceptably high levels of background fluorescence.
2.6. Invert coverslip on a glass slide and visualize cells fluorescence microscopy using the appropriate filters as soon as possible (Ex/Em = 484/501 nm).

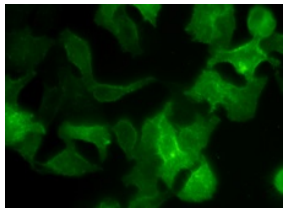

Fig. Hela cells stained with #A10020 Cell Plasma Membrane Staining Kit (Green Fluorescence).