

Product Data Sheet

Product Name: PKH 67 Cell Membrane Labeling Kit

Cat. No.: GC26306

Components

Solution A	PKH67 stock solution	0.1ml
Solution B	PKH67 dilution	10ml
Storage	-20°C, protect from light	

Protocol

This solution is for reference only. Please modify it according to your specific needs.

1. Preparation of staining solution:

(1) Before the first use, take the reagent out of the refrigerator and let it stand for a few minutes to return to room temperature. Centrifuge the tube of Solution A (PKH67 stock solution) briefly to fully concentrate the reagent at the bottom of the tube.

Note: Solution A is easy to degrade. It is recommended to divide Solution A according to the single use amount and store it at -20 or -80°C away from light to avoid repeated freezing and thawing.

(2) According to the number of cell samples to be tested, dilute Solution A 10 times with Solution B, and then dilute it 25 times with an appropriate solution (such as serum-free culture medium, HBSS or PBS) to prepare the staining working solution, which is ready for use.

Note: It is recommended to adjust the optimal working solution concentration according to different cells and experimental systems. The common scheme is to dilute Solution A 250 times for use. Some cells may need to adjust the concentration appropriately.

2. Cell staining

(1) Prepare a single cell suspension in a 1.5ml conical-bottom polypropylene centrifuge tube and wash the cells once with serum-free culture medium.

(2) Centrifuge the cells at 400×g for 5 minutes to obtain a loose cell pellet.

(3) Slowly aspirate the supernatant, being careful not to aspirate the cells.

(4) Use about 100μl of staining working solution to fully resuspend the cells. The recommended cell concentration in the working solution is about 10⁷/ml.

(5) Incubate at 2-8°C for 15-30 minutes. The optimal incubation time varies for different cells.

Note:

① It is recommended to incubate at 37°C for 5 minutes and then transfer to 4°C for 15 minutes. Low

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temperature incubation can reduce the endocytosis of the dye by the cells, help the dye to mark the cell membrane, and reduce the possibility of the dye localizing in the cytoplasmic vesicles.

② It is recommended to resuspend the cells 2-3 times during the staining process to help the dye fully label the sample.

(6) Centrifuge the cells at 20-25°C and 400×g for 10 minutes, slowly aspirate the supernatant, and then wash the cells 2-2 times with PBS or serum-free medium to ensure that the unbound dye is completely removed.

(7) Detection of labeled cells by flow cytometry, Ex/Em=490/502nm

3. Exosome Labeling

(1) Place the freshly isolated exosome pellet concentrate into a centrifuge tube, determine its volume, and bring the total volume to 1mL with Solution B.

(2) Add the same volume of exosome-free culture medium to a new centrifuge tube and bring the total volume to 1mL with Solution B to serve as a control.

(3) Add 6μL of Solution A to each tube. Mix continuously by gentle pipetting for 30s, then incubate at room temperature for 5 minutes. Add 2mL of 10% BSA (in PBS) to stop the reaction.

Note: It is recommended to adjust the optimal working fluid volume according to different exosomes and experimental systems

(4) Prepare a 0.971M sucrose solution. Add 1.5mL of this solution to the bottom of the tube, ensuring no turbulence is generated. The exosome-PKH67 solution will remain on top of the sucrose cushion. Centrifuge at 190,000g for 2 hours at 2-8°C.

Note: Exosomes will be present in the pellet, while most of the excess dye should remain at the interface layer.

(5) Carefully aspirate the medium and the interface layer. Gently resuspend the exosome pellet in PBS by pipetting. Transfer the suspension to an Amicon 10 kDa MWCO filter column and make up the volume to 9mL with PBS and 0.75mL of culture medium.

(6) Centrifuge at 3000g for 40 minutes to reduce the volume to 0.5-1mL.

(7) Recover the concentrated sample from the Amicon column and store it in a microcentrifuge tube

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on ice.

Notes

1. Fluorescent dyes all have quenching problems. Please try to avoid light during the experiment to slow down fluorescence quenching.
2. For your safety and health, please wear lab coats and disposable gloves when operating.

Background

PKH67 Cell Membrane Labeling Kit is a detection kit based on the green fluorescent probe PKH67. It can perform routine in vitro and in vivo cell membrane labeling and is suitable for studies such as cell proliferation and long-term in vivo cell tracking.

PKH67 is a membrane labeling probe with a longer aliphatic carbon tail in its structure, which can stably bind to lipid components in the cell membrane and emit fluorescence. PKH67 has the characteristics of low cytotoxicity, high lipid solubility, easy penetration of cell membranes, low fluorescence background after staining, and no staining of adjacent cells. The maximum excitation wavelength of PKH67 is 490nm, and the maximum emission wavelength is 502nm.

During the process of cell division and proliferation, the fluorescence intensity of PKH67 of the labeled cells will gradually weaken as the cells divide, and the labeled fluorescence can be evenly distributed to the two daughter cells, and the fluorescence intensity of the daughter cells is half of that of the mother cells. Based on this feature, flow cytometry can be used to study cell proliferation, cell cycle estimation, and cell division. The PKH67 Cell Membrane Labeling Kit can detect up to six or more divisions.

PKH67 Cell Membrane Labeling Kit can be used for in vitro and in vivo cell tracing. The fluorescence expression of PKH67 in the labeled cells is stable, the labeling positive rate is over 98%, and the labeled cells have good morphology, which can effectively observe the in vitro induced differentiation of cells. Injecting the labeled cells into the body can effectively track the migration and differentiation of transplanted cells in living tissues. The fluorescence half-life of PKH67 in vivo is 10-12 days, and the tracking observation time can be up to several weeks.

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