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PKH 67

Cat. No.:	HY-D1421
CAS No.:	257277-27-3
Target:	Fluorescent Dye
Pathway:	Others
Storage:	-80°C, protect from light

BIOLOGICAL ACTIVITY

Description	PKH67 is a fluorescent cell binding dye with green fluorescence. PKH67 can stain the cell membrane and the Ex/Em is 490/502 nm. PKH67 is often used in combination with the non-specific red fluorescent dye PKH26 (Ex/Em=551/567 nm) to label cells, detect cell proliferation in vitro, and trace cells in vitro and in vivo[1][2].
In Vitro	1. Preparation of dyeing solution (1) Take out the PKH 67 reagent from the refrigerator, let it stand for a few minutes to reach room temperature, or after a while in a 37°C water bath, centrifuge the tube containing PKH 67. Please be sure to centrifuge the tube for a few minutes to allow the reagent to fully dissolve before opening the cap. The lid can only be opened after it falls into the bottom of the tube. (2) According to the number of cell samples to be detected, dilute the probe 10 times with diluent, and then dilute the PKH 67 solution 25 times with a suitable solution (such as serum-free medium, HBSS or PBS) to prepare Dyeing working solution. Please adjust the optimal working solution concentration according to different cells and your own experimental system. Generally, cells can be diluted 250 times according to the final concentration of the liquid in the kit. Some cells may need to increase the concentration appropriately. 2. Cell staining (1) Resuspend the prepared cells to be tested in 100μL of staining solution to a cell concentration of approximately 10^7/mL. In situ staining can also be performed, as long as the staining solution is enough to cover the cells. (2) Culture cells at 2-8 °C for 15-30 minutes. The optimal culture time is different for different cells. (Note: It is recommended that the cells to be labeled be incubated in the dyeing working solution at 37°C for 5 minutes, and then incubated at 4°C for 15 minutes. Incubation at low temperature can reduce the endocytosis of the dye by the cells and help the dye to bind to the plasma membrane. labeling and reduce the likelihood of the dye localizing to cytoplasmic vesicles). (3) After centrifugation, remove the supernatant, collect the cells, wash the cells 1-2 times with PBS or serum-free medium, and finally add PBS or serum-free medium to resuspend the cells. (4) Take 500μL cell suspension and detect it with flow cytometer. Ex/Em=490/502 nm. (5) Subsequently, the cells can be cultured according to the normal culture method. (6) The labeling effect can be directly observed under a fluorescence microscope, or the cell proliferation can be detected by flow cytometry after culturing for an appropriate period, or used for cell fluorescence tracing for other specific experimental purposes. Precautions (1) The stain concentration varies depending on the cell type and the number of cells in each well.

(2) The prepared PKH 67 liquid is very easy to hydrolyze. It is recommended to store it in separate packages and freeze and dry it at ⊠-20°C.
(3) PKH 67 working solution should be prepared for immediate use and cannot be prepared in advance, because PKH 67 will decompose when absorbing water and affect the dyeing effect.
(4) PKH 67 is easily hydrolyzed and will deteriorate quickly in aqueous solution. Please avoid contact with water during use. The working fluid is in contact with water within the permitted time range during the process of labeling cells.
(5) PKH 67 fluorescent dye is an alcoholic solution. It will solidify and stick to the bottom, wall or lid of the centrifugation tube at lower temperatures such as 4°C or ice bath. It will recover after being taken out of the refrigerator. After it reaches room temperature and becomes liquid, centrifuge it to the bottom of the tube before opening the lid. It can be used after immersing it in a 37°C water bath until it is completely dissolved.
(6) The passages or times that can be traced after labeling for different cell types vary greatly. Please conduct testing based on the actual situation or reference literature.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Immun Inflamm Dis. 2024 Mar;12(3):e1155.

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REFERENCES

[1]. Shi J, et al. A genome-wide CRISPR screen identifies WDFY3 as a regulator of macrophage efferocytosis. Nat Commun. 2022 Dec 24;13(1):7929.

[2]. He L, et al. Intelligent manganese dioxide nanocomposites induce tumor immunogenic cell death and remould tumor microenvironment[J]. Chemical Engineering Journal, 2023: 141369.

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