PKH67 Green Fluorescent Cell Linker Kit

Introduction

PKH67 Green Fluorescent Cell Linker Kit is a kit that uses the green fluorescent probe PKH67 for labeling cell membranes. PKH67 can bind to lipid regions of cell membranes and show fluoresce green. PKH67 is less toxic to cells, has a low fluorescence background, and does not stain neighboring cells, so it is widely used for cell tracing in vitro and in vivo. PKH67-labeled cells have good morphology and stable fluorescence expression, which can effectively observe the situation of cells in vitro; PKH67-labeled cells can also be used for in vivo observation for up to several weeks. Meanwhile, the fluorescence of PKH67 is evenly distributed to daughter cells with cell division, and the fluorescence signal is related to the cell cycle state. Based on this property, PKH67 can also be used to detect cell proliferation. PKH67 can also be used in combination with PKH26 for experiments such as labeling cells, detecting proliferation, and cell tracing.

Components and Storage

Components	K2409-250 T	K2409-2500 T
PKH67 stock solution	100 μL	1 mL
Dilution buffer	10 mL	60 mL

Store the kit at -20°C, stable for 1 year. The PKH67 stock solution needs to be stored away from light and moisture.

Protocol

1. **Preparation of PKH67 working solution:**

   1) Allow PKH67 stock solution to warm to room temperature before using, or heat it in a 37°C water bath for a while to melt all the liquid. Then centrifugation for a few seconds to allow the liquid to be fully pooled to the bottom of the tube.

***Note:** PKH67 stock solution may solidify at lower temperatures and stick to the wall and caps of the tube. Before using, the liquid must gather to the bottom of the tube before opening the lid.

   2) Calculate firstly the required PKH67 working solution volume according to the experimental needs. The PKH67 stock solution is 10-fold diluted in the dilution buffer, then continues to be 25-fold diluted in a suitable solution (such as PBS, HBSS, or serum-free medium) to make the PKH67 working solution. It means that the PKH67 stock solution is 250-fold diluted to obtain the working solution. PKH67 working solution needs to be prepared and used on the spot and cannot be stored.

*Note: The optimal concentration of the PKH67 working solution varies depending on the cell types.

2. **Staining：**

1) Harvest 1x10^6 cells, wash cells twice with PBS, and subsequently resuspend the cells with 100 μL of PKH67 working solution (cell density: 1x10^7 cells/mL).

*Note: Adherent cells can also be detected in situ, and the working solution should be sufficient to cover the cells.

2) Incubate at 2-8°C protected from light for 10-30 min. The optimal incubation time for different cells varies and can be adjusted according to the experiment.

*Note: Low-temperature incubation reduces cell endocytosis of the dye and facilitates dye labeling of cell membranes.

3) After the incubation, centrifuge to remove the working solution, wash cells with PBS 1-2 times, and then resuspend the cells with appropriate amounts of PBS or serum-free medium. At this point, the detection can be performed directly with fluorescence microscopy or flow cytometry (Ex/Em: 490/502 nm), or the culture can be continued according to the normal culture method of the cell.

Note

1. PKH67 is easy to be hydrolyzed, so PKH67 stock solution should try to avoid contact with water, and it is recommended to store in aliquots. PKH67 working solution needs to be prepared and used on the spot and cannot be stored.
2. PKH67 stock solution is a DMSO solution, which may solidify at a lower temperature and stick to the wall and cap of the tube. So, it is necessary to warm to room temperature, and then centrifuge for a few seconds to allow the liquid to gather to the bottom of the tube before using.
3. For your safety and health, please wear lab coats and gloves during the experiment.
4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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