PKH26 Red Fluorescent Cell Linker Kit

Introduction

PKH26 Red Fluorescent Cell Linker Kit is a kit that uses the red fluorescent probe PKH26 for labeling cell membranes. PKH26 can bind to lipid regions of cell membranes and show fluoresce red. PKH26 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. PKH26-labeled cells have good morphology and stable fluorescence expression, which can effectively observe the situation of cells in vitro; PKH26-labeled cells can also be used for in vivo observation for up to several weeks. Meanwhile, the fluorescence of PKH26 is evenly distributed to daughter cells with cell division, and the fluorescence signal is related to the cell cycle state. Based on this property, PKH26 can also be used to detect cell proliferation. PKH26 can also be used in combination with PKH67 for experiments such as labeling cells, detecting proliferation, and cell tracing.

Components and Storage

Components	K2410-25 T	K2410-250 T
PKH26	100 μL	1 mL
Diluent	50 mL	500 mL

Store the kit at -20°C, stable for 1 year. PKH26 should be stored away from light and moisture.

Protocol

1. Harvest 2x10^7 cells and wash with serum-free medium for 1-2 times.

***Note:**

1) Adherent cells can also be tested in situ with sufficient working solution to cover the cells. However, in order to obtain better staining results, it is recommended to use single-cell suspension.
2) Serum proteins and lipids can bind to the dye, so it is recommended to wash with a serum-free medium before staining.

2. Centrifuge at 400 g for 5 min to form a loose cell pellet. Then discard the supernatant carefully, leaving no more than 25 μL of supernatant.

***Note:**

1) For reproducible results, minimize the volume of the residual medium before resuspending the cell pellet with Diluent.
2) PKH26 dye cannot be added directly to the cell pellet, which can easily cause heterogeneous staining.

3. Add 1 ml of Diluent to the cell pellet and gently resuspend the cells to make a 2X cell suspension. Resuspend

cells with gentle pipetting to ensure complete dispersion. At the same time, be careful not to allow the cells to be stored in Diluent for long periods.

***Note:** The presence of normal salts can cause the dye to clump and substantially reduce the staining efficiency. So, it is necessary to resuspend the cells in the Diluent at the time dye is added, not in medium or buffered salt solutions.

4. Add 4 μL of PKH26 to 1 mL of diluent, and mix gently, to make the 2X staining solution (4×10^{-6} M).

5. Quickly add 1 mL of 2X staining solution to 1 mL of 2X cell suspension and immediately mix well. The final cell density is $1x10^{7}$ cells/mL and the PKH26 dye concentration is 2×10^{-6} M.

***Note:**

1) Because staining is instantaneous, fast mixing is important to get correct and reproducible results. Shaking and vortexing are not recommended.
2) Mix equal volumes of the 2X cell suspension and 2X staining solution, and do not add PKH26 dye directly to the 2X cell suspension.
3) Adjust 2X cell suspension and 2X staining solution to avoid staining in very small (<100 μL) or very large (>5 mL).
4) Because PKH26 is an ethanol solution, in order to reduce the effect of ethanol on cell viability, it is recommended that the ethanol concentration in the mixed system should not exceed 1-2%.
5) If the final concentration of PKH26 in the system is less than 2×10^{-6} M, the PKH26 provided in the kit can be diluted with 100% ethanol prior to step 4 to achieve the desired 2X concentration, followed by step 4 dilution in Diluent to obtain the 2X staining solution.

6. Incubate at 25°C in the dark for 2-5 min. Gently invert the tube at regular intervals to help the cells mix thoroughly.

***Note:** Because the staining is so rapid, continuing to extend the incubation time does not help much.

7. Stop the staining reaction by adding an equal volume of serum (2 mL) or an equal volume of 1% BSA, and incubate for 1 min to bind excess dye.

***Note:**

1) Serum is the recommended stop solution. If replaced with a complete medium containing serum, 10 mL will need to be added.
2) It is not recommended to terminate the reaction by adding Diluent or centrifugation.
3) Serum-free media or buffer salts are not recommended as these can clump the dye.

8. Centrifuge at 400 g for 10 min at 20-25°C. Then carefully aspirate the supernatant, resuspend the cell pellet with 10 mL of complete medium and transfer it to a new centrifuge tube. Centrifuge at 400 g for 5 min at 20-25°C, and wash twice with a complete medium to remove unbound dye.

***Note:**

1) Transfer the resuspended cells to a new centrifuge tube to reduce the effect of residual dye on the washing efficiency of the tube wall.
2) It is not recommended to use Diluent to wash cells.

9. After washing, cells were resuspended in 10 mL of complete medium to assess cell recovery, cell viability, and fluorescence intensity. Centrifugate and resuspend the cells to the desired viable cell concentration.

*Note:

1) Stained cells can be fixed with neutral formaldehyde, and the fluorescence intensity can be retained for at least 3 weeks in the dark.
2) The fluorescence intensity of stained cells is generally 100-1000 times brighter than background autofluorescence. Although the CV value of staining is dependent on the cell species, the fluorescence distribution should be as uniform and symmetrical as possible.

10. Detect the samples directly with a fluorescence microscope or flow cytometry (Ex/Em: 551/567 nm).

Note

1. PKH26 is an ethanol solution, in order to avoid solvent volatilization and incorrect dye concentration, PKH26 should be stored as tightly as possible.
2. The 2X staining solution should be prepared freshly every time and cannot be stored.
3. There must be no azide present during PKH26 staining.
4. The number of tracking passages or times for different cells varies greatly.
5. For your safety and health, please wear lab coats and gloves during the experiment.
6. For research use only. Not to be used in clinical diagnostic or clinical trials.

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