Lumiprobe
Life science solutions

PKH Cell Membrane Labeling Kits manual

Contents

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PKH Cell Membrane Labeling Kits manual

PKH cyanine dyes contain lipophilic groups, which allow their rapid and non-covalent incorporation into the membranes of almost any cell without influencing cell receptors or transmembrane proteins. The cells preserve their biological properties and ability to proliferate, which makes PKH dyes useful for studying plant and animal cells *in vivo* and *in vitro*. These dyes can also be used to study membrane vesicles.

This ready-to-use kit contains all necessary reagents to label cell membranes with PKH dyes for evaluating cell migration or proliferation.

Kit components

Kit component	Count											
	13201 100 uL dye, 1x buffer	23201 100 uL dye, 5x buffer	33201 500 uL dye, 1x buffer	43201 500 uL dye, 5x buffer	14201 100 uL dye, 1x buffer	24201 100 uL dye, 5x buffer	34201 500 uL dye, 1x buffer	44201 500 uL dye, 5x buffer	17201 100 uL dye, 1x buffer	27201 100 uL dye, 5x buffer	37201 500 uL dye, 1x buffer	47201 500 uL dye, 5x buffer
2484-100uL, PKH26 dye, 1 mM solution in isopropanol, 100 uL	1	1	5	5	—	—	—	—	—	—	—	—
K6150, PKH Dyes Diluent, 1x, 10 mL	5	—	25	—	5	—	25	—	5	—	25	—
K7150, PKH Dyes Diluent, 5x, 10 mL	—	1	—	5	—	1	—	5	—	1	—	5
2485-100uL, PKH2 dye, 1 mM solution in isopropanol, 100 uL	—	—	—	—	1	1	5	5	—	—	—	—
2801-100uL, PKH800 dye, 1 mM solution in isopropanol, 100 uL	—	—	—	—	—	—	—	—	1	1	5	5

Store at 4 °C. Warm to RT before use.

Shelf life 12 months.

Recommendations for using the kit

- Optimal concentrations of the dye and cells can vary depending on cell and study type, so evaluate cell viability, homogeneity, and fluorescence intensity after staining.
- Do not use azide-containing solutions when staining with PKH dyes.
- Staining is more homogeneous when cell suspension is used.

Protocol

Protocol for cell membrane labeling with PKH dyes for RAW264.7 adhesion culture, 1×10^6 cells/sample, the final concentration of PKH dye 2 µM, final volume 200 µL.

1. Prepare PKH dye solution immediately before staining. Add 1 µL of PKH dye solution (*PKH dye, 1 mM solution in isopropanol*) to 9 µL of 96% ethanol, and add 4 µL of the resulting solution to 100 µL of *PKH Dyes Diluent, 1x.*

   **1x PKH Dyes Diluent is available either in ready-to-use form (K6150, PKH Dyes Diluent, 1x) or as a 5x concentrate (K7150, PKH Dyes Diluent, 5x). To dilute 5x PKH Dyes Diluent, use sterile bidistilled water.*

2. Remove the cell culture from the surface with a scraper in Hanks' solution (HBSS). Count the cells in the sample. Add 3 mL of Hanks' solution, centrifuge at 400 x *g* for 6 min at room temperature.

   **Serum proteins and lipids also bind the dye, so it is recommended to wash the cells once with serum-free medium or phosphate buffer saline.*

3. Remove the supernatant with a pipette, and resuspend a necessary amount of cells (e. g. 1×10^6 cells) in 100 µL of *PKH Dyes Diluent, 1x*. Add 100 µL of *PKH dye* solution prepared in step 1. Pipette and allow to stand at room temperature for 5 min. The final *PKH dye* concentration in the cell solution is 2 µM.

   **To obtain reproducible results, minimize the volume of the supernatant before cell resuspending.*

**Do not leave cells in PKH Dyes Diluent for a long period.*

**Staining is almost instant, so rapid cell dispersion in the dye solution is important to produce bright homogeneous and reproducible labeling.*

4. Add 2 mL of fetal bovine serum to stop the reaction, and incubate for 1 min. Centrifuge at 400 x *g* for 10 min at room temperature.

   **To stop the reaction, do not use a serum-free medium or buffered salt solution that results in dye aggregates.*

5. Remove the supernatant, resuspend the cells in 5 mL of complete culture medium, and transfer them to a new tube. Take aliquots to evaluate cell viability with trypan blue. Centrifuge at 400 x *g* for 10 min at room temperature.

6. Resuspend the cells in a buffer for further analysis (microscopy, flow cytometry, etc.).

   **Stained cells can be fixed with 2% paraformaldehyde, and staining remains stable for at least 3 weeks if samples are protected from light.*

Recommendations for storage

PKH dye solution can be stored at room temperature or in a refrigerator protected from light. Check the solution for precipitation before use. If a precipitate is seen in the dye solution, slightly warm it in a water bath at 37 °C and ultrasonicate or vortex until redissolved.

PKH Dyes Diluent is delivered as a 1x or a 5x solution in a sterile container. Store in a refrigerator and adjust to room temperature immediately before use.

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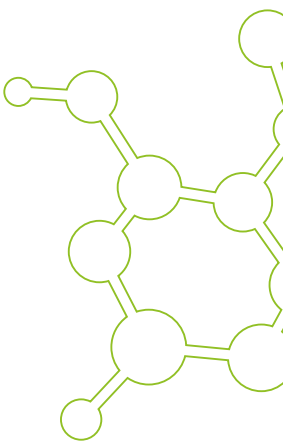

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