

## PKH Cell Membrane Labeling Kits manual



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## Kit components

Kit component	Count											
	13201	23201	33201	43201	14201	24201	34201	44201	17201	27201	37201	47201
100 µL dye, 1x buffer	100	100	500	500	100	100	500	500	100	100	500	500
2484-100µL, PKH26 dye, 1 mM solution in isopropanol, 100 µL	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
2485-100µL, PKH2 dye, 1 mM solution in isopropanol, 100 µL	—	—	—	—	1	1	5	5	—	—	—	—
2801-100µL, PKH800 dye, 1 mM solution in isopropanol, 100 µL	—	—	—	—	—	—	—	—	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,  $1 \times 10^6$  cells/sample, the final concentration of PKH dye  $2 \mu\text{M}$ , final volume  $200 \mu\text{L}$ .

1. Prepare PKH dye solution immediately before staining. Add  $1 \mu\text{L}$  of PKH dye solution (*PKH dye, 1 mM solution in isopropanol*) to  $9 \mu\text{L}$  of 96% ethanol, and add  $4 \mu\text{L}$  of the resulting solution to  $100 \mu\text{L}$  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  $3 \text{ mL}$  of Hanks' solution, centrifuge at  $400 \times g$  for  $6 \text{ min}$  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.  $1 \times 10^6$  cells) in  $100 \mu\text{L}$  of *PKH Dyes Diluent, 1x*. Add  $100 \mu\text{L}$  of *PKH dye* solution prepared in step 1. Pipette and allow to stand at room temperature for  $5 \text{ min}$ . The final *PKH dye* concentration in the cell solution is  $2 \mu\text{M}$ .

*\*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  $2 \text{ mL}$  of fetal bovine serum to stop the reaction, and incubate for  $1 \text{ min}$ . Centrifuge at  $400 \times g$  for  $10 \text{ min}$  at room temperature.

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

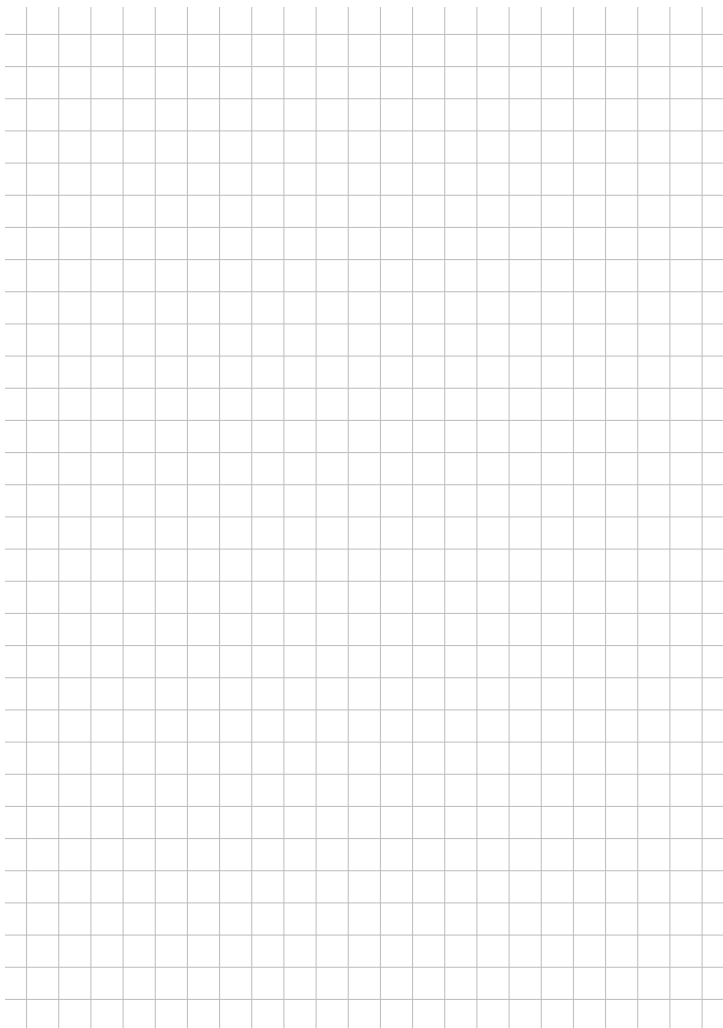
5. Remove the supernatant, resuspend the cells in 5 mL of complete culture medium, and transfer them to a new tube. Take aliquots to evaluate cell viability with trypan blue. Centrifuge at  $400 \times g$  for 10 min at room temperature.
6. Resuspend the cells in a buffer for further analysis (microscopy, 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.*

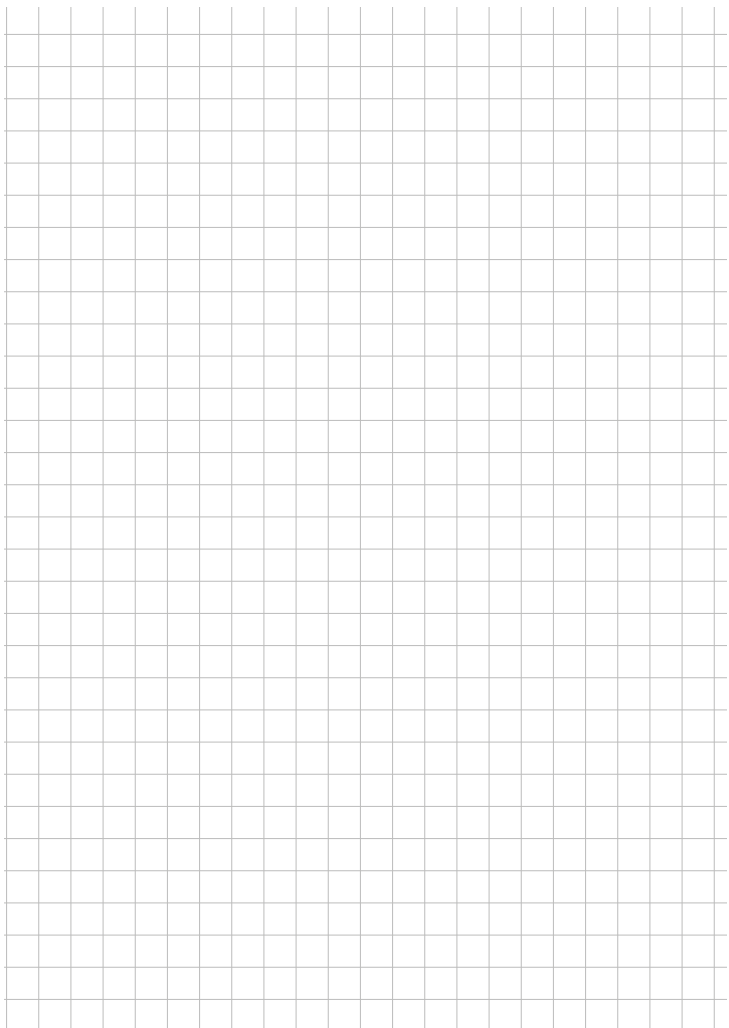
## Recommendations for storage

*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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