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# PKH67 Green Cell Membrane Labeling Kit

## Catalog Number: A069-1, A069-2

**Table 1. Kit Components and Storage**

| Material | Amount | Concentration | Storage | Stability |
|---|---|---|---|---|
| **PKH67 Green Cell Membrane Labeling Kit (Cat. No. A069-1)** | | | | The product is stable for at least one year when stored as directed. |
| PKH67 Dye (Component A) | 100 µL | 1 mM in DMSO | 2-8 °C<br>Protect from light | |
| Diluent C (Component B) | 50 mL | 1X | 2-8 °C | |
| **PKH67 Green Cell Membrane Labeling Kit (Cat. No. A069-2)** | | | | |
| PKH67 Dye (Component A) | 500 µL | 1 mM in DMSO | 2-8 °C<br>Protect from light | |
| Diluent C (Component B) | 5×50 mL | 1X | 2-8 °C | |

**Approximate fluorescence excitation/emission maxima, in nm:** 490/502.

### Product Description

The PKH67 Green Cell Membrane Labeling Kit uses proprietary membrane labeling technology to stably incorporate a green fluorescent dye with long aliphatic tails (PKH67) into lipid regions of the cell membrane. The labeling buffer provided in the kits (Diluent C) is an aqueous solution designed to maintain cell viability, while maximizing dye solubility and staining efficiency during the labeling step. Diluent C is iso-osmotic for mammalian cells and contains no detergents or organic solvents, but also lacks physiologic salts and buffers. The appearance of labeled cells may vary from bright and uniform to punctate or patchy, depending on the cell type being labeled.

PKH67 dye is well suited for cytotoxicity assays that use propidium iodide or 7-aminoactinomycin D as viability probes or for use in combination with orange-red fluorescent probes such as phycoerythrin, red fluorescent proteins, *etc*. It has also proven useful for monitoring exosome or liposome uptake, cell-cell membrane transfer, phagocytosis, and antigen presentation as well as for *in vivo* cell trafficking studies.

Correlation of *in vitro* cell membrane retention with *in vivo* rate of intensity decrease in non-dividing cells predicts an *in vivo* fluorescence half-life for PKH67 of 10-12 days. PKH67 is therefore recommended for short-to-medium term *in vivo* studies requiring a green cell linker dye, as well as for *in vitro* cytotoxicity, phagocytosis, proliferation, antigen presentation, or other co-culture assays. Due to its extremely stable fluorescence, PKH26 remains the cell linker dye of choice for longer term *in vivo* studies in which labeled cells are to be followed for periods longer than a few weeks.

### Recommendations for using the kit

1. 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.
2. Do not use azide-containing solutions when staining with PKH dyes.
3. Staining is more homogeneous when cell suspension is used.

### General Cell Membrane Labeling Protocol

The following labeling procedure can be used for *in vitro* or *ex vivo* labeling of stem cells, lymphocytes, monocytes, endothelial cells, neurons, or any other cell type where partitioning of dye into lipid regions of

the cell membrane is desired. Modified procedures may be required for *in vivo* labeling, for labeling platelets, or phagocytes.

General cell membrane labeling should be performed prior to monoclonal antibody staining. The membrane dyes will remain stable during the monoclonal staining at 4°C; however, capping of the monoclonal antibodies is highly probable if the general cell membrane labeling is carried out at ambient temperature after antibody labeling.

The cell and dye concentrations given in the following procedure represent starting concentrations that have been found broadly applicable to a variety of cell types. Users must determine the optimal dye and cell concentrations for their cell type(s) and experimental purposes by evaluating post-staining cell viability, fluorescence intensity, staining homogeneity, and lack of effect on cell function(s) of interest.

**Note:** Although adherent cells may be labeled while attached to a substrate, more homogeneous staining is obtained using single cell suspensions. Best results will be obtained if adherent or bound cells are dispersed into a single cell suspension using proteolytic enzymes, e.g., trypsin/EDTA, prior to staining.

**The following procedure uses a 500 µL of final staining volume containing final concentrations of 2 µM of PKH67 and 1 × $10^7$ cells/mL.**

Perform all further steps at ambient temperature

1. Place a suspension containing 2 × $10^7$ single cells in a conical bottom polypropylene tube and wash once using medium without serum.

Note: Serum proteins and lipids also bind the dye, reducing the effective concentration available for membrane labeling. Best results are obtained by washing once with serum-free medium or buffer (step 1) prior to resuspension in Diluent C for labeling (step 4).

2. Centrifuge the cells (400 × *g*) for 5 minutes into a loose pellet.
3. After centrifuging cells, carefully aspirate the supernatant, being careful not to remove any cells but leaving no more than 25 µL of supernatant.

Note: For reproducible results, it is important to minimize the amount of residual medium or buffer present when cells are resuspended in Diluent C.

4. Prepare a 2× Cell Suspension by adding 250 µL of Diluent C (Component B) to the cell pellet and resuspend with gentle pipetting to ensure complete dispersion. **Do not vortex and do not let cells stand in Diluent C for long periods of time**.

Note: The presence of physiologic salts causes the dye to form micelles and substantially reduces staining efficiency. Therefore, it is important that the cells be suspended in Diluent C at the time dye is added, not in medium or buffered salt solutions.

5. **Immediately prior to staining**, prepare a 2× Dye Solution (4 µM) in Diluent C by adding 1 µL of the PKH67 dye solution (Component A) to 250 µL of Diluent C in a polypropylene centrifuge tube and mix well to disperse.
6. **Rapidly add** the 250 µL of 2× Cell Suspension (step 4) to 1 mL of 2× Dye Solution (step 5) and **immediately mix** the sample by pipetting. Final concentrations after mixing the indicated volumes will be 1 × $10^7$ cells/mL and 2 µM PKH67.

Note: Because staining is nearly instantaneous, **rapid and homogeneous dispersion of cells in dye solution is essential for bright, uniform, and reproducible labeling**.

The following measures have been found to aid in optimizing results:

a. Do not add PKH67 dye directly to the 2× Cell Suspension in Diluent C.
b. Mix equal volumes of 2× Cell Suspension (step 4) and 2× Dye Solution (step 5).
c. Use a Pipetman or equivalent for rapid addition of cells and mixing with dye. Serological pipettes are slower and give less uniform staining. Mixing by “racking” or vortexing is also slower and gives less uniform staining.

7. Incubate the cell/dye suspension from step 6 for 1–5 minutes with periodic mixing. Because staining is so rapid, longer times provide no advantage.

Note: Expose cells to dye solution and Diluent C for the minimum time needed to achieve the desired staining intensity. Since Diluent C lacks physiologic salts, longer exposure may cause reduced viability in some cell types.

8. Stop the staining by adding 500 µL of 1% BSA solution and incubate for 1 minute to allow binding of excess dye.

Note: Do not stop by adding Diluent C or centrifuge the cells in Diluent C before stopping the staining reaction.

Note: **Do not use serum-free medium or buffered salt solutions**, which cause formation of cell-associated dye aggregates. Dye aggregates act as slow-release reservoirs of unbound dye that are not efficiently removed by washing and can transfer to unlabeled cells present in an assay.

9. Centrifuge the cells at 400 × g for 10 minutes at room temperature and carefully remove the supernatant, being sure not to remove cells. Resuspend cell pellet in 5 mL of complete medium, transfer to a fresh sterile conical polypropylene tube, centrifuge at 400 × g for 5 minutes at room temperature, and wash the cell pellet 2 more times with 5 mL of complete medium to ensure removal of unbound dye.

Note: Transfer to a fresh tube increases washing efficiency by minimizing carryover of residual dye bound to tube walls.

Note: **Do not use Diluent C for washing steps.**

10. After the final wash, resuspend the cell pellet in 5 mL of complete medium for assessment of cell recovery, cell viability, and fluorescence intensity. Centrifuge and resuspend to desired final concentration of viable cells.

Note: Stained cells may be fixed with 1-2% neutral buffered formaldehyde and intensities are stable for at least 3 weeks if samples are protected from light.

Note: Staining is typically at least 100-1,000 times brighter than background autofluorescence. Intensity distributions should be symmetrical and as homogeneous as possible, although staining CV will depend on the cell type being stained.