

PKH26 Exosome Labeling Kit

Introduction

Exosomes are a class of vesicle-like structures with a diameter of about 30-150 nm, which are released into the extracellular environment by cells through exocytosis. They are found in the body fluids of almost all living organisms, including blood, urine, saliva, milk, and cell supernatants cultured in vitro. Exosomes are important tools for cell-to-cell communication, which can carry and deliver a variety of biomolecules such as proteins, lipids, RNA, and DNA, thus playing an important role in regulating immune responses, promoting tissue repair, and transmitting genetic information.

PKH26 Exosome Labeling Kit is a kit that labels and traces exosomes through the PKH26 fluorescent probe. PKH26 can bind to lipid regions on exosome membranes and emit red fluorescence signals, thereby tracking exosomes in vitro and in vivo. This kit is optimized for better labeling. At the same time, this kit provides a labeling stop solution that can terminate PKH26 labeling. The PKH26-labeled exosomes can be used for subsequent cell experiments without purification.

Components and Storage

Size Components	50 Assays	250 Assays	Storage
PKH26 (250X)	20 μ L	100 μ L	-20°C away from light
Diluent C	200 μ L	1 mL	-20°C
Labeling Stop Solution	10 mL	50 mL	-20°C
Hoechst 33342 (1000X)	20 μ L	100 μ L	-20°C away from light
Shipping: Blue ice			

Protocol

1. Exosome quantification: Take some exosomes for protein quantification. We recommend using BCA Protein Assay Kit (Cat. No. K4101) for protein quantification. For exosomes extracted from cell culture media, we recommend using Exosome Isolation Kit for Cell Culture Media (Cat. No. K2707).

2. Preparation of PKH26 labeling working solution

- For 6-well plates, 100 μ L of PKH26 labeling working solution is required per well. Then calculates the volume of labeled working solution required for the experiment.

- 2) Dilute an appropriate amount of PKH26 (250X) with Diluent C at 1:10 ratio to obtain PKH26 (25X). Then dilute PKH26 (25X) 25-fold with a suitable buffer (must not contain BSA or serum) to obtain PKH26 labeling working solution.

***Note:**

- a) PKH26 is very easy to hydrolyze, so both PKH26 (25X) and PKH26 labeling working solution need to be prepared and used immediately.
- b) The optimal concentration of PKH26 labeling working solution can be adjusted between 0.5X-5X according to the specific experiment.
- c) Please prepare the PKH26 labeling working solution according to the recommended method. Do not directly add PKH26 (250X) to exosomes.

3. Label exosomes

- 1) Add 100 μL of PKH26 labeling working solution per 10 μg of exosomes for exosome labeling. After mixing, incubate at room temperature for 1-5 min in the dark. The recommended exosome volume should not exceed 10 μL .

***Note:**

- a) It is recommended to use high concentrations of exosomes ($>1 \text{ mg/mL}$) for experiments, as low concentrations may affect the results. When the exosome concentration is low, the concentration of PKH26 labeling working solution can be increased, which can be increased to 1.2X-5X.
- b) PKH26 staining is instantaneous, so the recommended incubation time should not exceed 10 min. Longer incubation may affect membrane integrity.
- c) The volume of PKH26 labeling working solution and exosomes can be adjusted according to the specific experiment, and avoid labeling exosomes in very small ($<100 \mu\text{L}$) or very large ($>5 \text{ mL}$) systems.

- 2) After incubation, add an equal volume of Labeling Stop Solution to the PKH26 labeling working solution, mix well, and incubate at room temperature for 1 min in the dark to terminate the remaining free dye. The volume of the system is approximately 200-220 μL .

***Note:** This termination reaction may be weak for some exosomes. In such cases, it is recommended to extract the exosomes again by exosome extraction or purification to remove the free PKH26 dye.

4. Exosome loading and tracking in cells

A. Adherent cells (using 6-well plates as an example)

- 1) Remove the medium, wash the cells once with PBS.
- 2) Mix the labeled exosomes (approximately 200-220 μL) with fresh serum-containing complete medium in a 1:9 ratio. Then add the mixture to cells.
- 3) Culture the cells in an incubator for 1-24 h. The incubation time can be adjusted according to the specific experiment.
- 4) After incubation, remove the medium and wash the cells twice with PBS.

- 5) If nuclear staining is required, perform the step 5. If there is no other need, add 2 mL of PBS directly and image using a fluorescence microscope. If using a microplate reader, it is recommended to use a black cell culture plate for detection. For flow cytometry testing, first digest cells into a single-cell suspension and then refer to the suspension cell procedure.

B. Suspension cells

- 1) Harvest 1×10^5 - 10×10^5 cells, centrifuge at 600 g at room temperature for 5 min, and discard the supernatant.
- 2) Mix the labeled exosomes (approximately 200-220 μ L) with fresh serum-containing complete medium in a ratio of 1:9. Then resuspend the cells with the mixed solution and add cells to the 6-well plate.
- 3) Culture the cells in an incubator for 1-24 h. The incubation time can be adjusted according to the specific experiment.
- 4) After incubation, centrifuge cells at 4°C, 600 g for 3-4 min. Discard the supernatant, being careful not to aspirate the cell pellet.
- 5) Wash the cells twice: Resuspend cells with 1 mL of PBS, centrifuge at 4°C, 600 g for 3-4 min, then remove the supernatant.
- 6) If nuclear staining is required, proceed to the step 5. If there is no other need, resuspend the cells directly with PBS and detect using a microscope, microplate reader, or flow cytometry.

5. Nuclear staining (optional)

- 1) Dilute an appropriate amount of Hoechst 33342 (1000X) with PBS at a 1:1000 ratio to obtain Hoechst 33342 (1X).
- 2) Add 1 mL of Hoechst 33342 (1X) to each well and incubate at room temperature for 10 min in the dark.
- 3) Discard Hoechst 33342 (1X) and wash the cells twice with PBS.

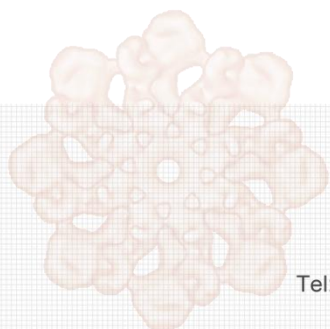
6. Detection

- 1) Proceed to detection for PKH26 (Ex/Em=551/567 nm) and Hoechst 33342 (Ex/Em=346/460 nm).

Notes

1. PKH26 (250X) is easy to solidify at low temperature and adhere to the wall or lid of the centrifuge tube. Please warm to room temperature in advance, and then centrifuge for 3-5 s to collect the liquid to the bottom of the tube.
2. Serum, BSA, or BSA analogues can interfere with PKH26 staining and should be avoided.

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3. Fluorescent probes are easy to quench, please protect them from light when using.
 4. For your safety and health, please wear lab coats and gloves during the experiment.
 5. For research use only. Not to be used in clinical diagnostic or clinical trials.



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