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PKH 67

Cat. No.:	HY-D1421
CAS No.:	257277-27-3
Target:	Fluorescent Dye
Pathway:	Others
Storage:	-80°C, protect from light

BIOLOGICAL ACTIVITY

Description

PKH67 is a fluorescent cell binding dye with green fluorescence. PKH67 can stain the cell membrane and the Ex/Em is 490/502 nm. PKH67 is often used in combination with the non-specific red fluorescent dye PKH26 (Ex/Em=551/567 nm) to label cells, detect cell proliferation in vitro, and trace cells in vitro and in vivo[1][2].

In Vitro

Cell labeling protocol for efferocytosis assay (Jurkat cells, for example)[1]:
1.Induce cell apoptosis with 5 μg/mL Staurosporine (HY-15141) in RPMI-1640 medium for 3 h at a density of 2.5×10^6 cells/mL at 37°C, 5% CO^2.
2.Wash Jurkat cells in 1X DPBS, and resuspend cells at a concentration of 2×10^7 cells/mL in Diluent C with either PKH67 (green fluorescence) or PKH26 (red fluorescence).
3.Rinse cells twice with DMEM basal medium containing 10% HI-FBS. Perpared cells should be immediately used for efferocytosis assay.
Protocol of this product is as follows:

1. Preparation of PKH 67 working solution
1.1 Preparation of the stock solution
Dilute PKH 67 masterbatch with preheated Diluent C at 1:500, i.e., dilute 2 μL of PKH 67 masterbatch with 1 mL of Diluent C.
Note: Please adjust the concentration of PKH 67 working solution according to the actual situation and use it now.
2. Cell staining
2.1 Suspension cells (6-well plate)
a.Centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant. Wash twice with PBS, 5 minutes each time.The cell density is 1×10^6/mL.
b. Add 1 mL of working solution, and then incubate at room temperature for 10-45 minutes.
c. Centrifuge at 400 g at 4°C for 3-4 minutes and then discard the supernatant.
d. Wash twice with PBS, 5 minutes each time.
e. Resuspend cells with serum-free cell culture medium or PBS. Observation by fluorescence microscopy or flow cytometry.
2.2 Adherent cells
a. Culture adherent cells on sterile coverslips.
b. Remove the coverslip from the medium and aspirate excess medium.
c. Add 100 μL of working solution, gently shake it to completely cover the cells,and then incubate at room temperature for 5-30 minutes.
d. Wash twice with medium, 5 minutes each time. Observation by fluorescence microscopy or flow cytometry.
3. Exsome Staining
a. Add 5-10 μL of dye working solution to the exosome with 200 μg protein/150 μL PBS.

b. Gently shake to completely cover the exosome surface and incubate for 30-60 min at room temperature and protected from light.
c. The fluorescently labeled exosomes are prepared by ultrafiltration or chromatography to remove the excess dye.
*Ultrafiltration: Purification by centrifugation through 100 kD ultrafiltration tubes at 20-25 ℃, 7000-10000 g, 15 min, repeated 4 times, and collection of precipitate.
*Centrifugation column chromatography: The column chromatography conditions were Sephadex G-25 Filler, PBS buffer (pH 7.4) as mobile phase; centrifugation conditions were 20-25 ℃, 1000-2000 g for 1-3 min, and the precipitate was collected.
d. Collect the purified sample, which is the labeled exosome.
4. Precautions 4.1 Please adjust the concentration of PKH 67 working solution according to the actual situation.
4.2 This product is for R&D use only, not for drug, household, or other uses.
4.3 For your safety and health, please wear a lab coat and disposable gloves to operate.
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Shi J, et al. A genome-wide CRISPR screen identifies WDFY3 as a regulator of macrophage efferocytosis. Nat Commun. 2022 Dec 24;13(1):7929.

[2]. He L, et al. Intelligent manganese dioxide nanocomposites induce tumor immunogenic cell death and remould tumor microenvironment[J]. Chemical Engineering Journal, 2023: 141369.

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