User Manual

DiI-Membrane

EVs Labeling & Purification Kit

(red)

Cat. # EXOPDiI10-1
EXOPDiI20-1

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Contents

Storage and Application

【Storage】

The DiI-Membrane EVs Labeling & Purification Kit (red) is shipped on ice, the components should be stored at recommended temperatures and protected from light. Properly stored kits are stable for 6 months. please read the instructions before use.

【Application】

For research use only. Not for use in diagnostic procedures.

Product Description

The carbocyanine dye DiI poses highly lipophilic nature and is widely used to stain cell membranes. The dye uniformly labels cells via lateral diffusion in the plasma membrane. The fluorescence of free DiI is very weak, but greatly enhanced when incorporated into membranes. DiI emits orange-red fluorescence when excited, its maximum excitation and emission wavelength is **549nm** and **565nm**, respectively.

Extracellular vesicles (EVs) are membrane-derived particles surrounded by a phospholipid bilayer that are released by cells. We use DiI dye to efficiently label EVs membranes. Then the excess unbound dyes are fast removed through our excellent Spin Columns from labeled exosome preparations, which separate molecules on the basis of the differences in size. Contaminant removal is easier and faster than traditional clean-up methods such as ultracentrifugation, spin filters.

With a highly specific membrane labeling and very low background levels, the kit can be used for most applications that require visualization of labeled EVs for tracking studies.

List of Components

Specification： **20** reactions/Kit (Cat.#EXOPDII20-1)； **10** reactions/Kit (Cat.# EXOPDII10-1)

Item (Cat.#EXOPDII20-1)	Volume	Storage Temp.
DiI Labeling Dye*	100μL	-20℃
Reaction Buffer	1.0mL	2-8℃
Spin Columns# within Collections Tubes	20 sets	2-8℃
1.5mL Light-proof Microcentrifuge Tubes	20 tubes	RT
Item (Cat.#EXOPDII10-1)	**Volume**	**Storage Temp.**
DiI Labeling Dye*	50μL	-20℃
Reaction Buffer	500μL	2-8℃
Spin Columns# within Collections Tubes	10 sets	2-8℃
1.5mL Light-proof Microcentrifuge Tubes	10 tubes	RT

*** Protect labeling dye from light.**

[#] **Keep the Spin Columns upright stand on end.**

The sterilized PBS buffer is not provided in the Kit. Please prepare at least 600uL PBS buffer for each reaction.

General Information

1. This Kit can label exosomes of any resources, including cell culture supernatants and body fluids (such as serum, plasma, urine, CSF or saliva).
2. When extracting exosomes for Labeling, the minimum dosage of serum and plasma is 500μL, the urine is 10mL, and the cell culture supernatant is 5mL.
3. It is not recommended to use exosomes extracted by PEG precipitation method, which contains too many impurities. The exosomes extracted by ultracentrifugation, affinity method or our company's Exosomes Extraction and Purification Kit is preferable.
4. Heat the dye at 37 ℃ until dissolved completely when the dye has crystallized.
5. Fluorescent dyes have quenching problems, please protect dyes from light during operating.
6. For your safety and health, please wear a lab gown and wear a disposable glove when operating.
7. The whole procedure is non-aseptic, please filter the labeled exosomes through 0.45um filter membrane before performing the downstream experiments.

Protocols

Staining Protocol

1. Vortex the DiI Labeling Dye and then instantaneous centrifugation, add 5μL DiI Labeling Dye to 50μL Reaction Buffer, and mix well until the dye is dissolved completely.

2. Add 50μL exosomes into the dye mixture slowly and mix well.

3. Incubate the mixture for 30 minutes at 37 ℃, pipet twice during the incubation period.

! Protect the tubes from light.

Purification Protocol (remove excess unbound dye)

4. Prepare the Spin Column prior to application of your sample.

a) Open the cap of the Spin Colum, aspirate preservative buffer from the top of the column with a micropipette and discard it, then remove the outlet plug of the column. In order to prevent drying of the column bed, proceed to the next step promptly.

b) equilibrate the column by adding 200 µL sterilized PBS buffer (not provided) and spin down at 100 x g for 90 seconds. If any PBS remains above the top frit, repeat spin at the same speed with 10 seconds increments. Discard the eluate.

c) Repeat the procedure b) again.

! Do not spin at too high speed or for too long as this may desiccate or compress the resin and decrease the function of spin column.

5. Carefully apply 100 µL exosome labeling preparation (from step 3 above) to the top of the column.

! The maximum capacity of the spin column is 100µL. Do not load samples more than 100µL.

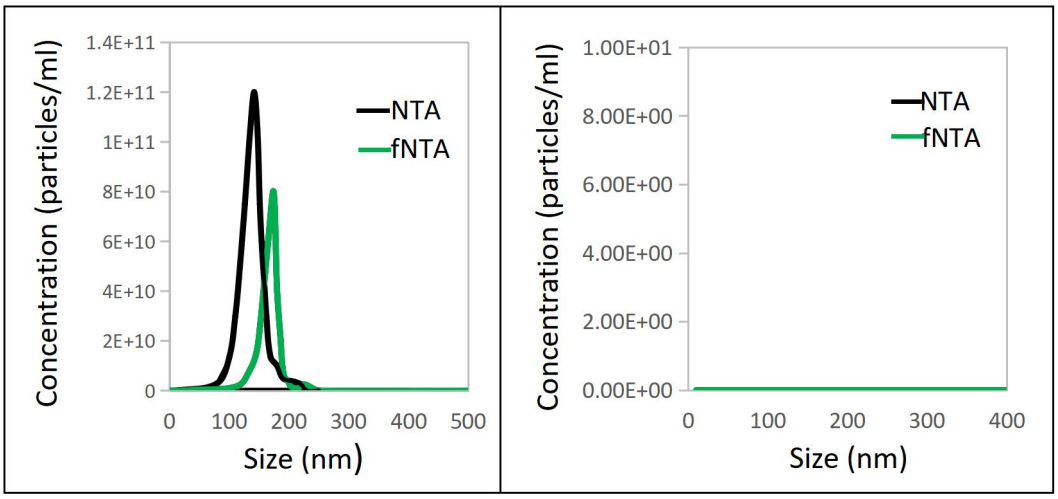
6. Centrifuge at 100 x g for 90 seconds. Discard the eluate.

7. Place the column into a fresh 1.5 mL Light-proof Microcentrifuge Tubes (provided). Apply 200 µL PBS buffer (not provided) to the top of the column.

8. Centrifuge at 100 x g for 90 seconds. The 200 µL eluate contains the labeled exosomes.

9. According to your needs, dilute the labeled exosomes with corresponding medium or not. Then filter the labeled exosomes through 0.45um filter membrane before performing the downstream experiments.

Example Data and Applications



A: DiI labeled EVs **B: Dye only (control)**

Figure 1. DiI labeled EVs and free dye control were analysed by NTA and fNTA.

The exosomes from LnCAP cell culture supernatant were concentrated by Exosome Concentration Kit (Cat# EXOCCon5-10), then were labeled and purified with DiI Membrane EVs Labeling & Purification Kit. A control involving dye but no exosomes was performed in parallel to confirm dye retention by the column. The results of NTA and fNTA indicated that a fairly considerable portion of exosomes were successfully labeled, the free dyes did not aggregate and the unbound dye can be removed successfully through the column.

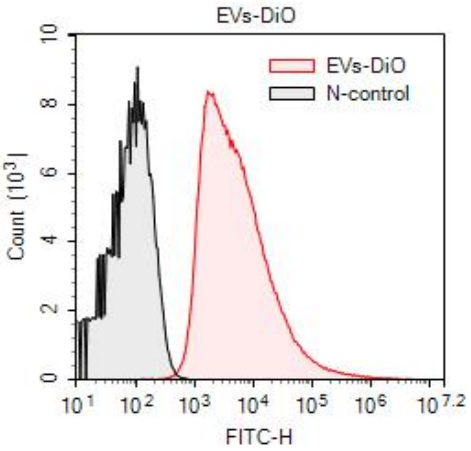


Figure 2. Flow cytometry detection of EVs from lnCAP cell culture supernatant.

We captured exosomes from 4.5mL LnCAP cell culture supernatant through magnetic beads coupled to CD63 antibodies. Then the bead-exosomes complexes were labeled with DiO and analyzed using the flow cytometry. As a control for unspecific binding of the dye to the beads, beads were stained with DiO without the addition of exosomes. The fluorescence intensity of DiO-labeled bead-exosomes (red lines) was significantly higher than that of control (black lines). The Kit enables efficient labeling of EVs.

Related Products

Exosome labeling & Purification	
DiO-Membrane EVs Labeling & Purification Kit (green)	EXOPDIO10-1/EXOPDIO20-1
DiR-Membrane EVs Labeling & Purification Kit (near-infrared red)	EXOPDIR10-1/EXOPDIR20-1
EVs labeling (DiO) Kit (green)	EXOPDIO10-1
EVs labeling (DiI) Kit (red)	EXOPDII10-1
EVs labeling (DiR) Kit (near-infrared red)	EXOPDIR10-1
Exosome Spin Columns (MW 4000)	RGESC12-1/ RGESC24-1
Exosome Isolation & Purification	
Exosome Extraction & Purification Kits（for blood serum/plasma）	EXORG10SP-1/ EXORG30SP-1/
Exosome Extraction & Purification Kits（for cell culture media/urine）	EXORG10CU-1/ EXORG30CU-1/
Exosome Concentration Kits (for cell culture media/urine)	EXOCCon5-10/ EXOUCon5-10
Total Exosome Capture & Isolation Kits（for cell culture media/urine）	EXOMBoCU-10/EXOMBoCU-20
Exosome Nucleic Acid Extraction	
Exosome Extraction & DNA Isolation Kits（for blood serum/plasma）	EXODNA30A-1/ EXODNA50A-1
Exosome Extraction & DNA Isolation Kits（for cell culture media/urine）	EXODNA10B-1/EXODNA24B-1
Exosome Extraction & RNA Isolation Kits（for bloos serum/plasma）	EXORNA30A-1/EXORNA50A-1
Exosome Extraction & RNA Isolation Kits（for cell culture media/urine）	EXORNA10B-1/EXORNA24B-1
Exo-Antibody	
Purified Anti-human Alix Antibody	RGAB100-50/RGAB100-100
Purified Anti-human CD9 Antibody	RGAB101-50/RGAB101-100
Anti-human CD9 Ab Biotin Conjugated	RGAB102-50/RGAB102-100
Purified Anti-human CD63 Antibody	RGAB103-50/RGAB103-100
Anti-human CD63 Ab Biotin Conjugated	RGAB104-50/RGAB104-100
Purified Anti-human CD81 Antibody	RGAB105-50/RGAB105-100
Anti-human CD81 Ab Biotin Conjugated	RGAB106-50/RGAB106-100

Troubleshooting

Q1： The fluorescence signal is low than expected?

A1： There may be too low amount exosome for labeling, it is recommended to take a larger sample to extract exosome. In addition, fluorescent dye will be quenched, please keep dye from light when operating. Ensure that the columns do not dry out during the procedure. Spinning the column for too long or at too high speed may cause the column to work inefficiently and loss of the labeled exosomes.

Q2： How to store the labeled exosomes when not carry out the downstream experiments immediately?

A2： The labeled exosomes can be stored at 2-8℃ for 1-2 days aware of light, If kept for a long time, it is recommended to store at -80℃ avoid repeated freezing and thawing.

Q3： Can I increase the elution volume?

A3： This is not recommended as it will result in co-elution of excess unbound dye with exosomes.

Q4： How I can know the counts of labeled exosomes?

A4： The labeled exosomes usually can be detected and counted by fluorescent Nanoparticle Tracking Analysis (fNTA) or flow cytometry. DiI labeling did not change the size of exosomes actually, but the calculation of particle size is related to many factors, which leads to the measurement value of size of the same labeled exosome sample under the fluorescence mode (fNTA) is larger than that under the conventional mode (NTA).

Technical Support

For more information about our products and to download manuals, please visit our web site: **http://www.rengenbio.com**

For additional information or technical assistance, please call or email us.

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