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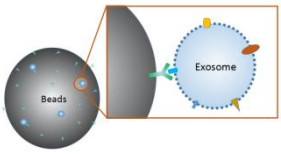

User Manual

Exosome Capture and Isolation Kits

for Cell Culture Media/Urine

Kit Name	Cat. Number
CD9 Kit	EXOMCUCD9-10
CD63 Kit	EXOMCUCD63-10
CD81 Kit	EXOMCUCD81-10
Combination Kit	EXOMCUCom-10

辽宁润基生物科技有限公司

Liaoning Rengen Biosciences Co.,Ltd

Version 1.0

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Contents

Storage and Application

【Storage】

The Exosome Capture and Isolation Kits is shipped on ice, the components should be stored at 2-8℃ upon receipt, **NOT FREEZEN**. Properly stored kits are stable for **6 MONTHS**. please read the instructions before use.

【Applications】

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

These kits are for the selective capture distinct sub-populations of exosomes based on particular surface markers. The isolated exosomes can be used for Western Blot, Transmission Electron Microscopy, Flow Cytometry, Labeling& Tracking and qRT-PCR etc.

Product Description

Exosome Capture and Isolation Kits is designed and optimized for isolation and enrichment of distinct sub-populations of exosomes from cell culture media or urine. Exosomes are extracellular vesicles secreted by most cell types and contain some microRNAs and surface marker proteins such as CD9, CD63, CD81. The kit consists of: 1μm **magnetic Capture Beads coupled with antibodies** that recognize exosome surface antigens CD9, CD63 or CD81, **Washing Buffer** and **Exosome Elution Buffer**.

The kit uses functionalized magnetic microparticles **(Capture Beads)** for exosome capture. The beads are modified with hydrophilic polymers to decrease non-specific binding and conjugated with antibody, which specifically binds to antigen of exosomes. The captured exosomes can be directly applied for Western Blot, Flow Cytometry and qRT-PCR etc, or gently eluted from magnetic beads for other applications such as NTA, Transmission Electron Microscopy and Labeling& Tracking etc..

Components per Kit

1. Specification: 10 reactions/Kit

Components	Volume	Storage Temperature
Each Capture Beads	1.0mL	2-8℃
Washing Buffer	30mL	2-8℃
Exosome Elution Buffer	0.5mL	2-8℃

2. Each kit contains these Capture Beads

Kit Name	Capture Beads	Characteristics
CD9 Kit	CD9 Capture Beads	Beads diameter: 1.0μm Solid content: 10mg/mL
CD63 Kit	CD63 Capture Beads	
CD81 Kit	CD81 Capture Beads	
Combination Kit	Mixture of CD9, CD63, CD81 Capture Beads.	

3. Test numbers by downstream application

Downstream Application	Test numbers*
Western blotting	20 tests
Flow cytometer analysis	20 tests
Nucleic acid detection (microRNA)	10 tests

*Test number is our recommendation. Customer may titrate beads amount according to sample target abundance.

Protocols

Protocol 1. Pre-Clearance Procedure

1. Dispense 20mL samples (cell culture media/urine) into 50mL tubes (not provided).
2. Centrifuge at 300 x g at 4° C for 10 minutes, and transfer supernatant to a new 50mL tubes (not provided).
3. Centrifuge the tubes at 2,000 x g at 4° C for 20 minutes, transfer supernatant to a new 50mL tubes(not provided).
4. (Optional) Filter the final supernatant with a 0.22μm filter unit.
5. The sample is now ready for immediate use with **Exosome Capture and Isolation Kits** or storage at -80° C.

Protocol 2. Exosome Capture Procedure

Example: 100μL beads*, 10mL cell culture media or 20mL urine

*Recommended beads amount for western blot sample preparation. You may titrate the Capture Beads amount according to your target abundance.

1. For urine, add 20ml Pre-Clearance sample and 20mL PBS (not provided) into a 50mL tube and mix upside down. For cell culture media, add 10mL Pre-Clearance sample directly into a 15mL tube.
2. Vortex Capture Beads well until the beads disperse completely, then add 100μL Capture Beads into each tube from step 1.
3. Incubate the sample for 3 hours at room temperature or 4° C overnight with gentle mixing. (Note: the optimal reaction time and temperature may depend on the target abundance and stability.)
4. Briefly spin the tube to collect beads from the top of the tube.

5. Place the tube on the magnetic stand (for 50mL tube) until all the beads collecting on one side of the tube or centrifuge the tube at 1,500 x g for 10 minutes and remove the supernatant.
6. Resuspend the beads with 1.0mL **Washing Buffer** and transfer whole this buffer containing Capture Beads to a fresh 1.5mL microfuge tube (not provided). Place the tube on the magnetic stand (for 1.5mL tube) until all the beads collecting on one side of the tube and remove the supernatant carefully.
7. Wash the beads with 1.0mL **Washing Buffer** again. Mix the beads briefly but thoroughly, then resuspend with 500µL **Washing Buffer**.
8. Place the tube on the magnetic stand (for 1.5mL tube) until all the beads collecting on one side of the tube and remove the supernatant carefully.

Protocol 3. Preparation Protocol for Western Blot (continued from protocol 2)

For Western Blot, you can add appropriate lysis buffer directly to the beads to extract exosomes proteins and conduct experiment according to the standard procedure of Western Blot with your preferred method/kit. We recommend **Exosomes Markers identification Kits (Western Blot)** (Rengen Biosciences CO., Cat.# EXOWBCD9-10, EXOWBCD63-10, EXOWBCD81-10, EXOWBAlix-10 or EXOWBTsg101-10) for exosomes markers identification.

Protocol 4. Preparation of exosome-bead complexes for flow cytometer analysis

(Continued from protocol 2).

1. Add 200µL of **Washing Buffer** to resuspend the beads and mix gently by pipetting (do not vortex) and utilize 100µL mixture for antibody labeling and flow cytometry analysis. Optimized volume of fluorescent antibody of a target or isotype control is added and incubated for 30 minutes at room temperature, pipet once each 5 minutes. (Note: titration of antibody conditions is important for optimal signal.)
2. Place the tube on the magnetic stand (for 1.5mL tube) and remove the supernatant.
3. Wash the beads 3 times with 200µL PBS (0.22um filtered) or your preferred buffer.

4. Add 100μL of PBS (0.22um filtered) or your preferred buffer to resuspend the beads. Then 20μL suspension solution is diluted with 180μL PBS (0.22um filtered) for flow cytometry.
5. Perform flow cytometer analysis.

Protocol 5. Exosome Capture Procedure for quantitative PCR (Continued from protocol 2).

Example : 200μL beads*, 20 ml sample

*Recommended Capture Beads amount for qPCR sample preparation. You may titrate the magnetic beads amount according to your target abundance. We recommend 20mL sample volume.

The Exosome RNA Isolation Kits (Rengen Biosciences CO., Cat.# EXORNA30C-1 /EXORNA50C-1) is available for nucleic acid isolation.

Obtained nucleic acids can be used as RT- qPCR template. Please follow supplier's instructions for cDNA synthesis reaction and qPCR reaction and use the isolated nucleic acids as much as possible if necessary.

Protocol 6. Exosome Elution Procedure(continued from protocol 2)

1. Add 50μL of **Exosome Elution Buffer** to resuspend the beads. Mix gently by pipetting.
2. Incubate the mixture for 10 minutes at room temperature. Vortex 3-5 times during the incubation.
3. Place the tube on the magnetic stand (for 1.5mL tube) for about 1 minute and transfer the supernatant to a fresh tube.

Note: It is highly recommended to warm up **Exosome Elution Buffer** at room temperature for at less 10 minutes and mix well until the solutions become clear again if turbid is present.

Related Products

Exosome labeling & Purification	
DiO-Membrane Exosome Labeling & Purification Kit (green)	EXODiO10-1/EXODiO20-1
DiI-Membrane Exosome Labeling & Purification Kit (red)	EXODiI10-1/EXOiI20-1
DiR-Membrane Exosome Labeling & Purification Kit (red)	EXODiR10-1/EXOiR20-1
PKH67-Membrane Exosome Labeling & Purification Kit (green)	EXOPKH67-10/EXOPKH67-20
Exosome Isolation & Purification	
Exosome Extraction & Purification Kits（for blood serum/plasma）	EXORG10SP-1/ EXORG30SP-1/
Exosome Concentration Kits (for cell culture media/urine)	EXOCCon5-10/ EXOUCon5-10
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 blood 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
Purified Anti-human TSG101 Antibody	RGAB107-50/RGAB107-100
Purified Anti-human PD-L1 Antibody	RGAB108-50/RGAB108-100
Anti-human PD-L1 Ab Biotin Conjugated	RGAB109-50/RGAB109-100
Purified Anti-humanEpCAMAntibody	RGAB110-50/RGAB110-100
Anti-human EpCAM Ab Biotin Conjugated	RGAB111-50/RGAB111-100

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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Wechat Public Platform

Exosome Research Exchange Group