Miltenyi Biotec

EV Isolation Kits
mouse

EV Isolation Kit CD9	130-117-042
EV Isolation Kit CD63	130-117-041
EV Isolation Kit CD81	130-117-040
EV Isolation Kit Pan	130-117-039

Contents

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1. Description

This product is for research use only.

Components

EV Isolation Kit CD9, mouse:
1 mL EV Isolation MicroBeads CD9, mouse
4 mL Equilibration Buffer
25 mL Isolation Buffer
20 µ Columns and plungers

or

EV Isolation Kit CD63, mouse:
1 mL EV Isolation MicroBeads CD63, mouse
4 mL Equilibration Buffer
25 mL Isolation Buffer
20 µ Columns and plungers

or

EV Isolation Kit CD81, mouse:
1 mL EV Isolation MicroBeads CD81, mouse
4 mL Equilibration Buffer
25 mL Isolation Buffer
20 µ Columns and plungers

or

EV Isolation Kit Pan, mouse:
1 mL EV Isolation MicroBeads Pan, mouse (cocktail of MicroBeads conjugated to CD9, CD63, and CD81)
4 mL Equilibration Buffer
25 mL Isolation Buffer
20 µ Columns and plungers

Capacity For 20 isolations.

Product format EV Isolation MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.

Storage Store EV Isolation MicroBeads, Equilibration Buffer, and Isolation Buffer protected from light at 2–8 °C. Do not freeze.
Store µ Columns and plungers dry at 10–35 °C.
The expiration dates are indicated on the labels.

1.1 Principle of the MACS Separation

The isolation of extracellular vesicles (EVs) like exosomes is performed by positive selection using MicroBeads recognizing the tetraspanin proteins CD9, CD63, or CD81. First, EVs are magnetically labeled during a short incubation period. The labeled EVs are loaded onto a µ Column, which is placed in the magnetic field of a µMACS™ Separator. The magnetically labeled EVs are retained within the column, while the unlabeled vesicels and cell components run through the column. After removing the column from the magnetic field, the intact EVs can either be collected by elution with Isolation Buffer, or directly lysed in the column and the protein in the lysates can be analysed, e.g., by Western blotting.

1.2 Background information

EVs, including exosomes, are secreted by virtually every cell type and can be found in all body fluids such as blood, urine, saliva, ascites fluid, and cerebrospinal fluid. EVs carry important molecules (e.g. RNAs, proteins, lipids) from their parent cells and are involved in many normal and pathological processes.
CD9, CD63, and CD81 are three of the most-studied members of the tetraspanin protein family and can be used to isolate EVs.

1.3 Applications

- Isolation of EVs from cell culture supernatant.
- Isolation of EVs from body fluids like plasma, urine, or ascites.

1.4 Reagent and instrument requirements

- µMACS Separator (# 130-042-602)
- MACS MultiStand (# 130-042-303)
- Centrifuge
- 1.5 mL tubes

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- (Optional) EV lysis and elution buffer for direct loading of EV proteins on sodium dodecyl sulfate (SDS) gels, e.g., 50 mM Tris-Cl, pH 6.8, with 2% (w/v) SDS, 8% (v/v) glycerin, and 0.005% (w/v) bromophenol blue.
- (Optional) Serum-free cell culture medium
- (Optional) EDTA or citrate tubes
- (Optional) Phosphate-buffered saline (PBS)
- (Optional) Filter with 0.22 µm nylon mesh

2. Protocol

▲ Kit components should not be substituted or mixed with components from other kits or lots.

2.1 Sample preparation

The EV Isolation Kits can be used for pre-cleared cell culture supernatant or pre-cleared body fluids like plasma, ascites, or urine as well as to isolate EVs carrying the respective markers from EV preparations, e.g., from ultracentrifugation or density gradient centrifugations.

Pre-clearing cell culture supernatant

1. Incubate the cells of interest in serum-free medium for 12–72 hours depending on the cell line. Adjust the incubation conditions to an apoptosis rate of less than 5%.
2. Remove cells, cell debris, and larger vesicles by serial centrifugations at 300×g for 10 minutes, 2000×g for 30 minutes, and 10,000×g for 45 minutes. Take off completely the respective supernatant for the next centrifugation step.
3. (Optional) Filter the supernatant through a filter with 0.22 µm nylon mesh.

Pre-clearing plasma

1. Add blood to EDTA or citrate tubes.
2. Separate plasma by centrifugation at 1,000×g for 10 minutes.
3. Dilute plasma with an equal volume of PBS.
4. Remove cell debris and larger vesicles by serial centrifugations at 2000×g for 30 minutes and 10,000×g for 45 minutes. Take off completely the respective supernatant for the next centrifugation step.

Pre-clearing urine or ascites

1. Remove cells, cell debris, and larger vesicles by serial centrifugations at 300×g for 10 minutes, 2000×g for 30 minutes, and 10,000×g for 45 minutes. Take off completely the respective supernatant for the next centrifugation step.
2. (Optional) Filter the supernatant through a filter with 0.22 µm nylon mesh.

2.2 Magnetic labeling

▲ Volumes for magnetic labeling given below are for up to 2 mL EV-containing sample such as pre-cleared cell culture supernatant, plasma, urine, asictes, etc.

1. Add 50 µL of EV Isolation MicroBeads to EV-containing sample and vortex.
2. Incubate for 1 hour at room temperature.
3. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Always wait until the column reservoir is empty before proceeding to the next step.

▲ Pre-warm Equilibration Buffer and Isolation Buffer to room temperature before use.

1. Place a µ Column in the magnetic field of the µMACS Separator that is attached to the MACS MultiStand.
2. Prepare column by applying 100 µL Equilibration Buffer on top of the column.
3. Rinse column with 3×100 µL of Isolation Buffer. Only add new buffer when the column reservoir is empty.
4. Apply magnetically labeled sample onto the column and let it run through.
5. Wash column with 4×200 µL of Isolation Buffer. Only add new buffer when the column reservoir is empty.
6. (Optional) Remove any residual drop at the column tip by touching the column tip with a pipette tip.
7. Proceed to elution of EVs (2.4).

2.4 Elution

Perform elution of EVs with column outside of the magnetic separator to obtain intact vesicles.

Elution of intact EVs

▲ Eluted EVs remain attached to MicroBeads and might interfere with downstream analysis like nanoparticle tracking analysis or electron microscopy as both, the EVs and MicroBeads, will be analyzed.

1. Remove the column from the magnetic separator and place column onto a 1.5 mL tube. Add 100 µL Isolation Buffer to the column and immediately flush out the magnetically labeled vesicles by firmly pushing the plunger into the column.
2. Eluted EVs can be used for, e.g., RNA isolation or Western blot analysis.

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Elution of EV lysate for Western blot analysis

1. Remove the column from the magnetic separator and place column onto a 1.5 mL tube. Add 100 µL EV lysis and elution buffer to the column and immediately flush out the vesicle lysate by firmly pushing the plunger into the column.
2. The lysed EV samples can directly be used for further Western blot analysis.

3. Troubleshooting

Slow column flow

▲ Air bubble formation within the column can impair column flow. To prevent air bubble formation, use room-temperature buffers for the wash steps or, where possible, degas the buffers before use.

No or low EV recovery

▲ EV binding to the EV Isolation MicroBeads mainly depend on the EV concentration. Low recoveries can be indicative for low EV concentration. Extended culture times could improve the yield. Alternatively, EVs can be pre-concentrated from larger volumes, e.g., by ultracentifugation.

▲ Vesicles might have no epitopes used for isolation. Although, most EV carry the tetraspanin markers CD9, CD63, or CD81, some EV populations have been shown to not equally carry all of the three markers.

▲ Columns are still magnetized. The µ Columns must be removed from the µMACS Separator to enable successful elution.

EVs still present in the flow through

▲ The binding capacity of the EV Isolation MicroBeads could be overloaded in case of very high concentration of EVs, e.g., from cell culture medium or pre-enriched EV samples. Repeat the experiment with diluted EV samples.

▲ Contaminations, e.g., from cell culture medium could interfere with EV binding to the EV Isolation MicroBeads. Repeat the experiment with diluted EV samples or use isolated EVs instead of cell culture supernatant.

Co-purification of negative EVs

▲ High concentration of EVs or contaminations, e.g., from cell culture medium can give rise to non-specific binding of EVs to the MicroBeads. Repeat the experiment with diluted EV samples or use isolated EVs instead of cell culture supernatant.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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