EasySep™ Human Extracellular Vesicle (CD9) Positive Selection Kit

For processing 20 mL of biofluid

Catalog #17894

Positive Selection

Document #10000005332 | Version 03

STEMCELL™ TECHNOLOGIES

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Description

Isolate human CD9+ extracellular vesicles (EVs) from plasma, serum, cell culture conditioned medium, and urine by immunomagnetic positive selection.

- Fast and easy-to-use
- No columns required

This kit targets EVs for positive selection with an antibody recognizing the specific tetraspanin marker CD9. Desired EVs are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted biofluid components are simply poured off, while desired EVs remain in the tube. The final isolated fraction contains highly purified EVs that are immediately available for downstream applications such as DNA/RNA extraction, western blot, or mass spectrometry.

NOTE: Antibody complexes and particles bound to EVs may interfere with Brilliant Violet™ antibody conjugates, polyethylene glycol-modified proteins, or other chemically related ligands.

NOTE: Following positive selection, particle release is not recommended for downstream applications that lyse EVs to analyze cargos (e.g. DNA/RNA extraction and western blot). If particles are released, EVs remain bound to antibody complexes.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human CD9 Positive Selection Cocktail	17894C	1 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.
EasySep™ Releasable RapidSpheres™ 50201	50201	2 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles in water.

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Sample Preparation

For available fresh and frozen samples, see www.stemcell.com/primarycells.

PLASMA (FROM WHOLE BLOOD)

NOTE: This kit is compatible with plasma samples derived from whole blood collected in common blood collection tubes (e.g. acid-citrate-dextrose, EDTA, and heparin) or specialized preservation tubes (e.g. Nucleic Acid Plus BCT® [Streck Catalog #230698], Cell-Free DNA BCT® [Streck Catalog #218961], RNA Complete BCT® [Streck Catalog #230460], PAXGene Blood DNA Tubes [BD Biosciences Catalog #761115], and PAXGene Blood RNA Tubes [BD Biosciences Catalog #762165]). For more information regarding collection tubes, contact us at techsupport@stemcell.com.

1. Centrifuge whole blood at 2000 - 2500 x g for 10 - 15 minutes with the brake off. Remove the plasma layer and transfer to a conical tube (e.g. Catalog #38009/38010).
2. Centrifuge the plasma layer (from step 1) at 2000 - 2500 x g for 10 - 15 minutes with brake off. Remove the plasma supernatant and transfer to a new conical tube.
3. OPTIONAL: To remove cellular debris and large vesicles, centrifuge plasma supernatant at 10,000 x g for 30 minutes. Remove plasma supernatant and transfer to the required tube or plate (see Tables 1 - 2).

CONDITIONED MEDIUM

1. Harvest conditioned medium from cell culture and transfer to a conical tube (e.g. Catalog #38009/38010).
2. Centrifuge the conditioned medium at 2000 x g for 10 minutes. Remove the supernatant and transfer to a new conical tube.
3. OPTIONAL: To remove cellular debris and large vesicles, centrifuge supernatant at 10,000 x g for 30 minutes. Remove the supernatant and transfer to the required tube or plate (see Tables 1 - 2).
4. OPTIONAL: If starting with diluted samples, concentrate using tangential flow filtration (TFF) filter (e.g. TFF-Easy [Catalog #100-2460]) or centrifugal filter tube (30 kDa or 100 kDa).

URINE

If using frozen urine, thaw fully before processing the sample.

1. Vortex urine to obtain a homogenous suspension.
2. Centrifuge urine at 1000 x g for 10 minutes at room temperature (15 - 25°C). Remove supernatant and transfer to a new tube.

   NOTE: For fresh urine, we recommend adding protease inhibitors to prevent protein degradation.

   NOTE: If not used immediately, store urine in accordance with current laboratory guidelines for urine sample handling.
3. OPTIONAL (RECOMMENDED): Pre-clear sample by centrifuging supernatant at 10,000 x g for 30 minutes at room temperature.

   NOTE: This step will reduce THP contamination, but may lower final EV recovery.
4. For samples ≤ 2 mL, remove supernatant and transfer to the required tube or plate (see Tables 1 - 2),

   OR

   For samples > 2 - 20 mL, transfer urine to a 100K centrifugal filter tube to concentrate the sample. Centrifuge at 1000 x g for 30 minutes at room temperature. Collect retained volume above filter membrane and transfer to the required tube (see Table 1). Top up to 1 mL with recommended medium.

For other biofluids, follow protocol for conditioned medium (steps 2 - 3) to remove cells and large vesicles.

Recommended Medium

D-PBS (Without Ca++ and Mg++; Catalog #37350)

Directions for Use – Manual EasySep™ Protocols

See pages 1 and 2 for Sample Preparation and Recommended Medium. Refer to Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human Extracellular Vesicle (CD9) Positive Selection Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	**EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)**
1	**Add sample to required tube.**	≤ 0.5 - 2 mL	1 - 8 mL
	Required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Add Selection Cocktail to sample.** **NOTE: Do not vortex cocktail.**	50 µL/mL for samples ≥ 0.5 mL NOTE: For samples < 0.5 mL, add 25 µL of cocktail.	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes OR RT for 30 minutes for concentrated urine samples	RT for 10 minutes OR RT for 30 minutes for concentrated urine samples
3	**Vortex Releasable RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
4	**Add Releasable RapidSpheres™ to sample.**	100 µL/mL for samples ≥ 0.5 mL NOTE: For samples < 0.5 mL, add 50 µL of particles.	100 µL/mL of sample
	Mix and incubate.	RT for 10 minutes OR RT for 30 minutes for concentrated urine samples	RT for 10 minutes OR RT for 30 minutes for concentrated urine samples
5	**Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.**	Top up to 2.5 mL	• Top up to 5 mL for samples < 4 mL • Top up to 10 mL for samples ≥ 4 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
6	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant.** **NOTE: Do not remove the tube from the magnet between separations.**	Discard supernatant	Discard supernatant
7	**Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.**	Top up to 2.5 mL	• Top up to 5 mL for samples < 4 mL • Top up to 10 mL for samples ≥ 4 mL
	Incubate.	RT for 1 minute	RT for 1 minute
8	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant.** **NOTE: Do not remove the tube from the magnet between separations.**	Discard supernatant NOTE: If starting sample is conditioned medium, skip to step 10.	Discard supernatant NOTE: If starting sample is conditioned medium, skip to step 10.
9	**Repeat steps as indicated.**	Steps 7 and 8, two more times (total of 1 x 5-minute and 3 x 1-minute separations)	Steps 7 and 8, two more times (total of 1 x 5-minute and 3 x 1-minute separations)
10	**Remove the tube from the magnet. Resuspend EVs in desired medium. Be sure to collect the EVs from the sides of the tube.**	Isolated EVs are ready for use	Isolated EVs are ready for use

RT - room temperature (15 - 25°C)

* Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.

Table 2. EasySep™ Human Extracellular Vesicle (CD9) Positive Selection Kit Protocol

STEP	INSTRUCTIONS	EasyPlate™ (Catalog #18102)
1	Add sample to required plate.	0.2 mL
2	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample NOTE: For samples < 0.2 mL, add 10 µL of cocktail.
	Mix and incubate.	RT for 10 minutes OR RT for 30 minutes for concentrated urine samples
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Add Releasable RapidSpheres™ to sample.	100 µL/mL of sample NOTE: For samples < 0.2 mL, add 20 µL of RapidSpheres™.
	Mix and incubate.	RT for 10 minutes OR RT for 30 minutes for concentrated urine samples
5	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 0.2 mL
	Place the plate (without lid) into the magnet and incubate.	RT for 5 minutes
6	Carefully pipette (do not pour) off the supernatant. NOTE: Do not remove the plate from the magnet between separations.	Discard supernatant
7	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 0.2 mL
	Incubate.	RT for 1 minute
8	Carefully pipette (do not pour) off the supernatant. NOTE: Do not remove the plate from the magnet between separations.	Discard supernatant NOTE: If starting sample is conditioned medium, skip to step 10.
9	Repeat steps as indicated.	Steps 7 and 8, two more times (total of 1 x 5-minute separation and 3 x 1-minute separations)
10	Remove the plate from the magnet. Resuspend EVs in desired medium.	Isolated EVs are ready for use

RT - room temperature (15 - 25°C)

Directions for Use – Custom Protocols

Contact us at techsupport@stemcell.com to obtain:

- Automated protocol and appropriate RoboSep™-S bottle for fully automated RoboSep™-S and RoboSep™-16 positive selection of EVs
- Manual protocol and recommended buffer for particle release following EV positive selection

Notes and Tips

For assessment of CD9 tetraspanin marker by western blot immunostaining, use the following enzyme or fluorochrome-conjugated antibody clone:

- Anti-Human CD9 Antibody, Clone HI9A (Catalog #100-0138)

For more information, refer to the web protocol: How to Characterize Extracellular Vesicles by Western Blotting, available at www.stemcell.com.

BIOFLUID VARIABILITY

Types and levels of tetraspanin expression on EVs within and between biofluid samples can be variable. This may affect isolation yields and tetraspanin data obtained in subsequent analyses.

OPTIMIZING RECOVERY

To improve EV recovery, use one of the following methods:

- Increase Selection Cocktail incubation time to 30 minutes, and/or
- Double the volumes of Selection Cocktail and RapidSpheres™
  NOTE: The volumes of the two components must be increased at the same time.
- For samples < 2 mL, use of EasySep™ Magnet (Catalog #18000) is recommended.

Data

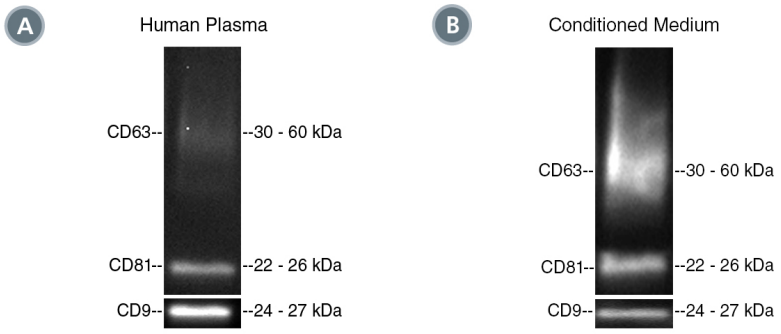

Figure 1. Representative Western Blot Analysis of CD9+ EVs Isolated from Human Plasma and Conditioned Medium

The western blot analyses in the above examples show positive isolation of EVs with the CD9 tetraspanin marker from (A) human plasma and (B) conditioned medium.

References

Welsh, J. A. et al. (2024). Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. J Extracell Vesicles 13. Epub ahead of print, DOI:10.1002/jev2.12404.

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