EasySep™ Extracellular Vesicle PE Positive Selection Kit

For processing 20 mL of biofluid or conditioned medium

Catalog #100-0812

Positive Selection

Document #10000012044 | Version 00

STEMCELL™ TECHNOLOGIES

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TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713

INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM

FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Description

Isolate human extracellular vesicles (EVs) labeled with PE (phycoerythrin)-conjugated antibodies from plasma, serum, urine, and cell culture conditioned medium by immunomagnetic positive selection.

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

This kit targets EVs labeled with PE-conjugated antibodies (not provided) for positive selection. Desired EVs are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted components are simply poured off, while desired EVs remain in the tube. Following positive selection, **particles should not be released from EVs**. 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.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Extracellular Vesicle PE Positive Selection Cocktail	300-0443	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 and 0.1% BSA.
EasySep™ Releasable RapidSpheres™ 50201	50201	4 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.

BSA - bovine serum albumin; 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)

1. Centrifuge whole blood at 2000 x g for 10 minutes. 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 x g for 10 minutes. Remove the plasma supernatant and transfer to a new conical tube.
3. Centrifuge plasma supernatant at 10,000 x g for 30 minutes to remove cellular debris and large vesicles. Remove plasma supernatant and transfer to the required tube or plate (see Tables 1 - 2).

   OPTIONAL: If desired, plasma can be filtered using a 0.2 µm filter prior to isolation of EVs.

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

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. 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 a 30K or 100K centrifugal filter tube (e.g. PALL Catalog #MAP030C36/MAP100C36).
5. OPTIONAL: If desired, supernatant can be filtered using a 0.2 µm filter prior to isolation of EVs.

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, freeze urine at -20°C for long-term storage.
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.

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 - 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Extracellular Vesicle PE Positive Selection Kit Protocol

		EASYSEP™ MAGNETS	
STEP	**INSTRUCTIONS**	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	**Prepare sample within the volume range.**	0.5 - 2 mL	1 - 8 mL
	Add sample to 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 PE-conjugated antibody to sample.**	1 µg/mL of sample NOTE: For samples < 0.5 mL, add 0.5 µg of antibody.	1 µg/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
3	**Add Selection Cocktail to sample.** **NOTE: Do not vortex cocktail.**	50 µL/mL of sample NOTE: For samples < 0.5 mL, add 25 µL of cocktail.	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
4	**Vortex Releasable RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
5	**Add Releasable RapidSpheres™ to sample.**	200 µL/mL of sample NOTE: For samples < 0.5 mL, add 100 µL of RapidSpheres™.	200 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
6	**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
7	**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
8	**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
9	**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 11	Discard supernatant NOTE: If starting sample is conditioned medium, skip to step 11
10	**Repeat steps as indicated.**	Steps 8 and 9, two more times (total of 1 x 5-minute and 3 x 1-minute separations)	Steps 8 and 9, two more times (total of 1 x 5-minute and 3 x 1-minute separations)
11	**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™ Extracellular Vesicle PE Positive Selection Kit Protocol

STEP	INSTRUCTIONS	EasyPlate™ (Catalog #18102) 
1	Add sample to required plate.	0.2 mL
2	Add PE-conjugated antibody to sample.	1 µg/mL of sample NOTE: For samples < 0.2 mL, add 0.2 µg of antibody.
	Mix and incubate.	RT for 15 minutes
3	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 3 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
5	Add Releasable RapidSpheres™ to sample.	200 µL/mL of sample NOTE: For samples < 0.2 mL, add 40 µL of RapidSpheres™.
	Mix and incubate.	RT for 3 minutes
6	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
7	Carefully pipette (do not pour) off the supernatant. NOTE: Do not remove the plate from the magnet between separations.	Discard supernatant
8	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
9	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 11
10	Repeat steps as indicated.	Steps 8 and 9, two more times (total of 1 x 5-minute separation and 3 x 1-minute separations)
11	Remove the plate from the magnet. Resuspend EVs in desired medium.	Isolated EVs are ready for use

RT - room temperature (15 - 25°C)

Notes and Tips

For assessment of CD63, CD81, and CD9 tetraspanin markers by western blot immunostaining, use Extracellular Vesicle Human CD9/CD63/CD81 Antibody Panel (Catalog #100-0211) or the following antibody clones:

- Anti-Human CD9 Antibody, Clone HI9A (Catalog #100-0138), and
- Anti-Human CD63 Antibody, Clone H5C6 (Catalog #100-0139), and
- Anti-Human CD81 (TAPA-1) Antibody, Clone 5A6 (Catalog #100-0209)

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

BIOFLUID VARIABILITY

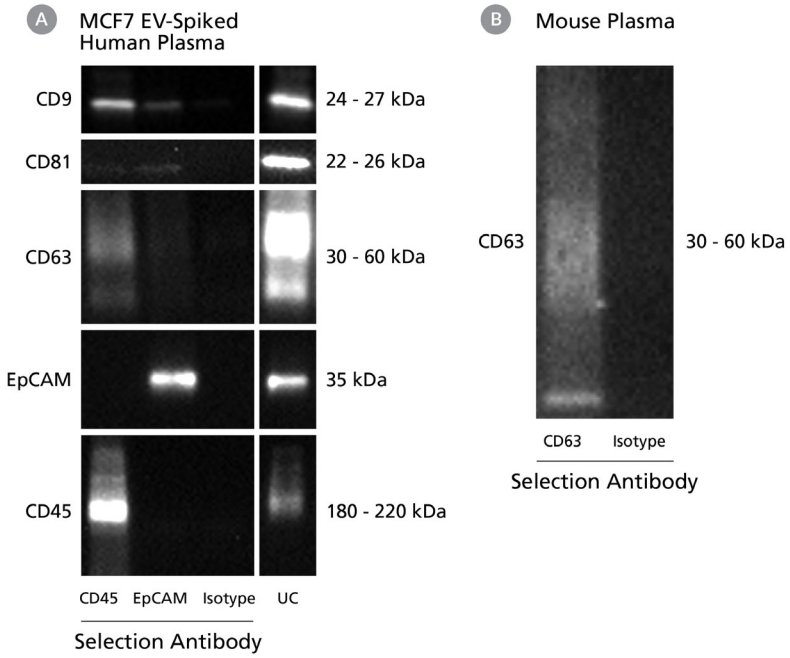
Types and levels of tetraspanin expression on EVs within and between biofluid samples can be variable. This may affect isolation yields and tetraspanin data obtained in subsequent analyses.

OPTIMIZING RECOVERY

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

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

Data



(A) EVs were isolated from healthy human plasma spiked with cancer cell (MCF7)-derived EVs by differential ultracentrifugation (UC) or using the following PE-conjugated antibodies with EasySep™ Extracellular Vesicle PE Positive Selection Kit: Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018PE); anti-human EpCAM antibody, clone 9C4; and Mouse IgG1, kappa Isotype Control Antibody, Clone MOPC-21 (Catalog #60070PE). Target markers (EpCAM and CD45) and common EV markers (CD9, CD81, and CD63) were analyzed by western blot under non-reducing conditions.

(B) EVs were isolated from mouse plasma using PE-conjugated anti-mouse CD63 antibody, clone NVG-2 or PE-conjugated rat IgG2a isotype control antibody with the EasySep™ Extracellular Vesicle PE Positive Selection Kit. Target marker (CD63) was analyzed by western blot under non-reducing conditions.