EasySep™ Human Pan-Extracellular Vesicle Positive Selection Kit

For processing 20 mL of biofluid

Catalog #17891

Positive Selection

Document #10000005292 | Version 02

STEMCELL™ TECHNOLOGIES

Scientists Helping Scientists™ | WWW.STEMCELL.COM

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) from plasma, serum, and cell culture conditioned medium by immunomagnetic positive selection.

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

This kit targets EVs for positive selection with antibodies recognizing the specific tetraspanin markers CD9, CD63, and CD81. 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. 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™ Human Pan-Extracellular Vesicle Positive Selection Cocktail	17891C	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)

1. Centrifuge whole blood at 2000 x g for 10 minutes. Remove the plasma layer and transfer to a new tube.
2. Centrifuge the plasma layer (from step 1) at 2000 x g for 10 minutes. Remove the plasma supernatant and transfer to a new 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 (see Table 1).

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

CONDITIONED MEDIUM

1. Harvest conditioned medium and transfer to a 50 mL conical tube (e.g. Catalog #38010).
2. Centrifuge the conditioned medium at 2000 x g for 10 minutes. Remove the supernatant and transfer to a new tube.
3. Centrifuge supernatant at 10,000 x g for 30 minutes. Remove the supernatant and transfer to the required tube (see Table 1).

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

Recommended Medium

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

Directions for Use – Manual EasySep™ Protocols

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

Table 1. EasySep™ Human Pan-Extracellular Vesicle 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	RT for 10 minutes
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	RT for 10 minutes
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; no need for repeated separations.	Discard supernatant NOTE: If starting sample is conditioned medium, skip to step 10; no need for repeated separations.
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.

Notes and Tips

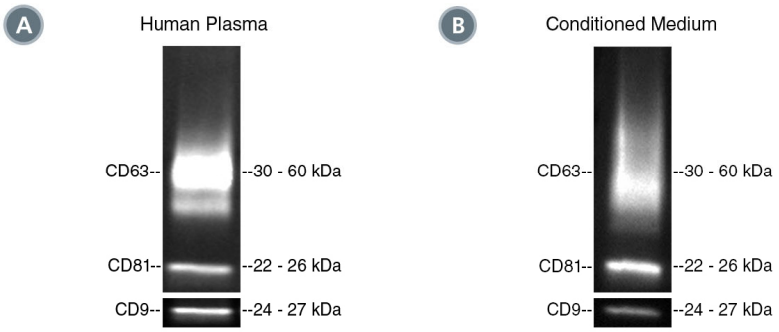
For assessment of CD63, CD81, and CD9 tetraspanin markers by Western blot immunostaining, use the following enzyme or fluorochrome-conjugated antibody clones:

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

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.

Data



The Western blot analyses in the above examples show positive isolation of EVs with CD9, CD63, and CD81 tetraspanin markers from (A) human plasma and (B) conditioned medium.