EasySep™ Human Extracellular Vesicle (CD61) Depletion and Positive Selection Kit

For processing 20 mL of biofluid

Catalog #100-2079

Depletion and Positive Selection

Document #10000030228 | Version 01

STEMCELL™ TECHNOLOGIES

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Description

Deplete or isolate platelet-derived extracellular vesicles (EVs) from plasma or serum by immunomagnetic depletion or positive selection. Differential ultracentrifugation-purified EV preparations can also be used for depletion of platelet-derived EVs.

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

This kit targets platelet-derived EVs for depletion or positive selection with an antibody recognizing the human platelet membrane glycoprotein IIIa (Integrin beta-3) CD61. For depletion, unwanted CD61+ EVs are labeled with antibodies and magnetic particles and separated without columns using an EasySep™ magnet (Tables 1 and 2). Samples depleted of CD61+ EVs are simply poured off into a new tube and are immediately available for downstream applications, such as flow cytometry and ELISA. Samples depleted of CD61+ EVs can be subsequently processed to isolate other EV subtypes (see Notes and Tips).

A separate protocol allows for positive selection of CD61+ EVs (Tables 3 - 5). Desired CD61+ 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 CD61+ EVs remain in the tube. The final isolated fraction contains CD61+ 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 positively selected CD61+ 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 Extracellular Vesicle (CD61) Depletion and Positive Selection Cocktail	300-1160	1 x 0.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in D-PBS.
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.

D-PBS - Dulbecco's 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/human-peripheral-blood-products.html

PLASMA (FROM WHOLE BLOOD WITH ANTICOAGULANT) or SERUM (FROM WHOLE BLOOD WITHOUT ANTICOAGULANT AFTER CLOTTING)

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. 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 brake off. Transfer the upper layer (leaving about 1 cm above the red blood cell pellet) to a conical tube (e.g. Catalog #38009/38010).
2. Centrifuge the upper layer (from step 1) at 2000 - 2500 x g for 10 - 15 minutes with brake off. Transfer the supernatant (leaving about 1 cm above the red blood cell pellet) to a new conical tube.

   OPTIONAL: To remove residual platelets, filter plasma or serum using a 0.8 µm filter (e.g. Millipore ATTP02500) prior to EV isolation.

   OPTIONAL: Processed plasma or serum can be frozen as per the latest guidelines from the International Society for Extracellular Vesicles.

   OPTIONAL: To remove large vesicles, further centrifuge plasma or serum supernatant at 10,000 x g for 30 minutes. Transfer supernatant to a new conical tube.
3. Transfer processed plasma or serum to the required tube or plate (see Tables 1 - 5).

DIFFERENTIAL ULTRACENTRIFUGATION-PURIFIED EV PREPARATION

NOTE: If desired, serum or plasma EVs can be prepared by differential ultracentrifugation (dUC) prior to CD61+ EV depletion. It is not recommended to perform dUC after EV depletion.

1. dUC-purified EV preparations should be generated as per a user-selected protocol (e.g. Théry et al.). For example, dUC-purified EV preparations can be generated by two ultracentrifugation steps at 100,000 x g and 4°C for 70 minutes each, using a Type 45 Ti fixed-angle rotor at maximum acceleration and deceleration.
2. EV preparations should be adjusted to less than 9 x 10^10 particles/mL. If particle concentration is not available, final volume can be adjusted to a maximum of 30% of the initial volume of plasma or serum used in preparation.

Recommended Medium

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

Directions for Use – Manual EasySep™ Protocols

See page 2 for Sample Preparation and Recommended Medium. Refer to Tables 1 - 2 (depletion) or Tables 3 - 5 (positive selection) for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human Extracellular Vesicle (CD61) DEPLETION 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 EasySep™ Cocktail to sample.** **NOTE: Do not vortex cocktail.**	25 µL/mL of sample	25 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
3	**Vortex Releasable RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
4	**Add Releasable RapidSpheres™ to sample.**	200 µL/mL of sample	200 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	**Mix by gently pipetting up and down 2 - 3 times. 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 into a new tube.**	Samples are ready for use	Samples 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 (CD61) DEPLETION Protocol

		EASYSEP™ MAGNETS	
		EasyEights™ (Catalog #18103)	
STEP	INSTRUCTIONS	5 mL tube	14 mL tube
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 EasySep™ Cocktail to sample. NOTE: Do not vortex cocktail.	25 µL/mL of sample	25 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
3	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add Releasable RapidSpheres™ to sample.	200 µL/mL of sample	200 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	Mix by gently pipetting up and down 2 - 3 times. Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
6	Carefully pipette** (do not pour) the supernatant into a new tube.	Samples are ready for use	Samples are ready for use

RT - room temperature (15 - 25°C)
** Collect the entire supernatant, all at once, into a single pipette (e.g. for EasyEights™ 5 mL tube, use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube, use a 10 mL serological pipette [Catalog #38004]).

Table 3. EasySep™ Human Extracellular Vesicle (CD61) POSITIVE SELECTION 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 EasySep™ Cocktail to sample. NOTE: Do not vortex cocktail.	25 µL/mL of sample	25 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
3	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add Releasable RapidSpheres™ to sample.	200 µL/mL of sample	200 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 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 down2 - 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	Discard supernatant
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.	Samples are ready for use	Samples 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 4. EasySep™ Human Extracellular Vesicle (CD61) POSITIVE SELECTION Protocol

		EASYSEP™ MAGNETS	
		EasyEights™ (Catalog #18103)	
STEP	INSTRUCTIONS	5 mL tube	14 mL tube
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 EasySep™ Cocktail to sample. NOTE: Do not vortex cocktail.	25 µL/mL of sample	25 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
3	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add Releasable RapidSpheres™ to sample.	200 µL/mL of sample	200 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 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	Carefully pipette** (do not pour) the supernatant into a new tube. 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 2 minutes	RT for 2 minutes
8	Carefully pipette** (do not pour) the supernatant into a new tube. NOTE: Do not remove the tube from the magnet between separations.	Discard supernatant	Discard supernatant
9	Repeat steps as indicated.	Steps 7 and 8, two more times (total of 1 x 5-minute and 3 x 2-minute separations)	Steps 7 and 8, two more times (total of 1 x 5-minute and 3 x 2-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.	Samples are ready for use	Samples are ready for use

RT - room temperature (15 - 25°C)
** Collect the entire supernatant, all at once, into a single pipette (e.g. for EasyEights™ 5 mL tube, use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube, use a 10 mL serological pipette [Catalog #38004]).

Table 5. EasySep™ Human Extracellular Vesicle (CD61) POSITIVE SELECTION Protocol

STEP	INSTRUCTIONS	EasyPlate™ (Catalog #18102) 
1	Prepare sample within the volume range.	0.05 - 0.2 mL
	Add sample to required plate.	96-Well Round-Bottom Microplate (e.g. Catalog #38018)
2	Add EasySep™ Cocktail to sample. NOTE: Do not vortex cocktail.	5 µL
	Mix and incubate.	RT for 5 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Add Releasable RapidSpheres™ to sample.	40 µL
	Mix and incubate.	RT for 3 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 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
9	Repeat steps as indicated.	Steps 7 and 8, two more times
10	Remove the plate from the magnet. Resuspend EVs in desired medium.	Samples are ready for use

RT - room temperature (15 - 25°C)

Directions for Use – Custom Protocols

Contact us at techsupport@stemcell.com to obtain:

- Custom protocol and appropriate RoboSep™-S bottle for fully automated RoboSep™-S depletion or positive selection of CD61+ EVs
- Custom protocol and appropriate buffer for particle release after CD61+ EV positive selection
- Dual protocol to perform sequential depletion and positive selection of CD61+ EVs from the same sample

Notes and Tips

For assessment of CD61+ EV depletion or positive selection, use the following antibody clone:

- Anti-human CD61 antibody, clone VI-PL2 or clone PM6/13

For assessment of CD9, CD63, and CD81 tetraspanin markers by western blot, 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.

EV ISOLATION AFTER DEPLETION OF CD61+ EVs

Samples depleted of CD61+ EVs can be further processed for isolation of other EV subtypes using size-exclusion chromatography (SEC) columns (e.g. Catalog #100-0415), immunomagnetic selection using another EasySep™ kit (e.g. EasySep™ Human Pan-Extracellular Vesicle Positive Selection Kit [Catalog #17891], or marker-specific isolation using EasySep™ Extracellular Vesicle PE Positive Selection Kit [Catalog #100-0812]). Isolated samples are immediately available for downstream applications, such as DNA/RNA extraction, western blot, or mass spectrometry.

BIOFLUID VARIABILITY

Types and levels of CD61 and tetraspanin expression on EVs can be variable within and between biofluid samples. This may affect isolation yields and tetraspanin data obtained in subsequent analyses.

Data

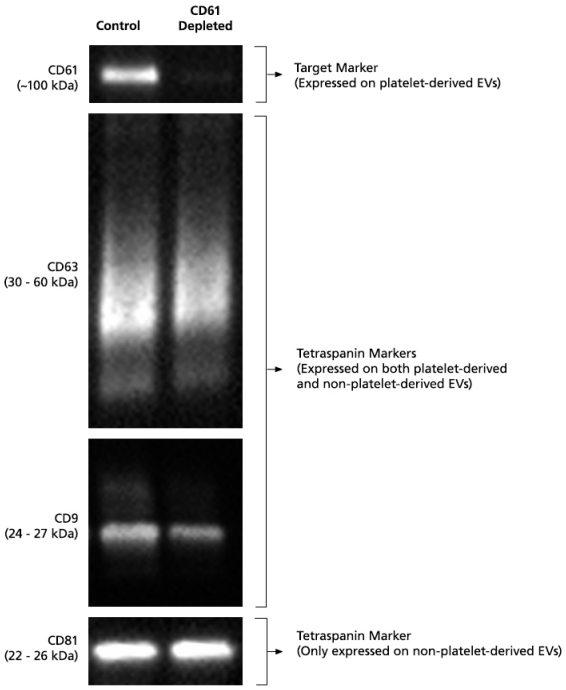

Figure 1. Representative Western Blot Analysis of Human Plasma Depleted of CD61+ EVs.

Starting with processed plasma from normal healthy donors, CD61+ EVs were first depleted using the EasySep™ Human Extracellular Vesicle (CD61) Depletion and Positive Selection Kit. EVs from CD61-depleted plasma or control plasma (no depletion) were subsequently isolated using the EasySep™ Human Pan-EV Positive Selection Kit. Samples were analyzed by western blot under non-reducing conditions for the expression of CD61 (the target marker), CD9, CD63 (expressed on both platelet-derived and non-platelet-derived EVs), and CD81 (not expressed on platelet-derived EVs; Koliha et al.). The CD61 signal intensity was quantified from the same image at the same exposure time for EVs from both the depleted and control plasma. On average, 95.7 ± 3.5% (mean ± SD using the purple EasySep™ magnet) of CD61+ EVs were depleted compared to the control. In the above example, 95% of CD61+ EVs were depleted compared to the control.

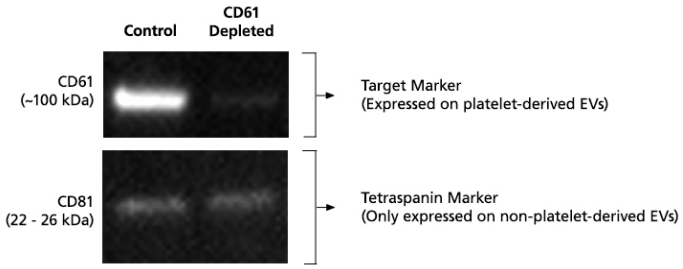

Figure 2. Representative Western Blot Analysis of dUC-Purified EV Preparations Depleted of CD61+ EVs.

Starting with dUC-purified EV preparations from human plasma of normal healthy donors, CD61+ EVs were depleted using the EasySep™ Human Extracellular Vesicle (CD61) Depletion and Positive Selection Kit and compared to the control EV preparation (no depletion). Samples were analyzed by western blot under non-reducing conditions for the expression of CD61 (the target marker) and CD81 (not expressed on platelet-derived EVs; Koliha et al.). The CD61 signal intensity was quantified from the same image at the same exposure time for both the depleted and control samples. On average, 95.7± 4.9% (mean ± SD using the purple EasySep™ magnet) of CD61+ EVs were depleted compared to the control. In the above example, starting with 1 mL of dUC-purified EV preparation containing 8.6 x 10^10 extracellular particles, 85% of CD61+ EVs and 5.2 x 10^10 of extracellular particles were depleted based on western blot and Spectradyne® nCS1 analyses (size range 72 - 400 nm, transit time < 100 µs), respectively.

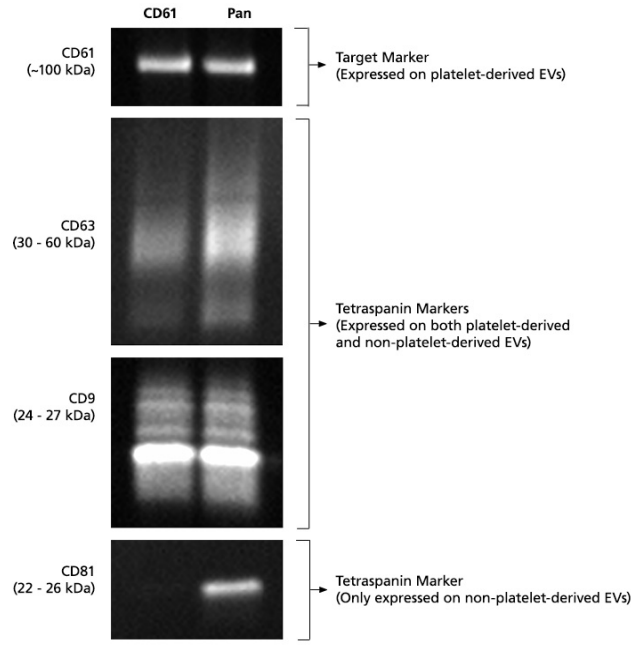

Figure 3. Representative Western Blot Analysis of CD61+ EVs Isolated from Human Plasma.

Starting with human plasma from normal healthy donors, EVs were isolated using either the EasySep™ Extracellular Vesicle (CD61) Depletion and Positive Selection Kit or the EasySep™ Human Pan-EV Positive Selection Kit. CD61 (the target marker), CD9, CD63 (expressed on both platelet-derived and non-platelet-derived EVs), and CD81 (not expressed on platelet-derived EVs) were analyzed by western blot under non-reducing conditions. In the above example, the isolated CD61+ EVs demonstrated the expected phenotype of platelet-derived EVs (CD61+, CD63+, CD9+, and CD81-).

References

Koliha N et al. (2016) A novel multiplex bead-based platform highlights the diversity of extracellular vesicles. J Extracell Vesicles 5: 29975.

Théry C et al. (2006) Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr Protoc Cell Biol 3(3): 22.