

EasySep™ Human Pan-Extracellular Vesicle Positive Selection Kit

For processing 20 mL of urine

Catalog #17891

Positive Selection

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Description

Isolate human extracellular vesicles (EVs) from urine 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 urine 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

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 (see Table 1).

OR

For samples > 2 - 20 mL, transfer urine to a 100K centrifugal filter tube (e.g. PALL Catalog #MAP100C36) 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 page 1 for Sample Preparation and Recommended Medium. Refer to Table 1 for detailed instructions regarding the EasySep™ procedure.

Table 1. EasySep™ Human Pan-Extracellular Vesicle Positive Selection Kit Protocol for URINE

STEP	INSTRUCTIONS	EasySep™ (Catalog #18000)	
1	Prepare sample within the volume range.	0.5 - 2 mL	
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	
2	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	
	Mix and incubate.	RT for 10 minutes OR RT for 30 minutes for concentrated samples	
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	
4	Add RapidSpheres™ to sample.	100 µL/mL of sample	
	Mix and incubate.	RT for 10 minutes OR RT for 30 minutes for concentrated 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	
	Place the tube (without lid) into the magnet and incubate.	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	
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	
	Incubate.	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	
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 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	

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

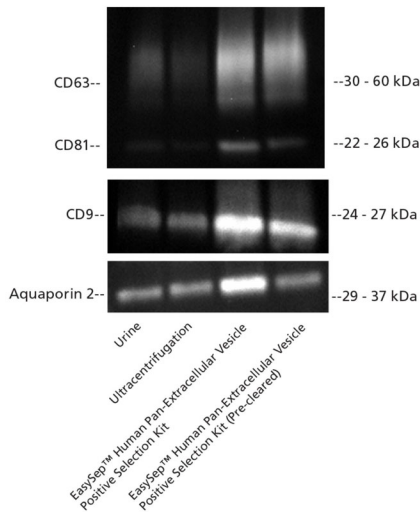
For assessment of CD63, CD81, and CD9 tetraspanin markers by western blot immunostaining, use the following enzyme or fluorochrome-conjugated antibody clones:

- 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 urine samples can be variable. This may affect isolation yields and tetraspanin data obtained in subsequent analyses.

Data



The western blot analysis in the above example shows positive expression of CD9, CD63, CD81, and Aquaporin 2 on EVs isolated from human urine. From left to right, EVs were isolated from 1 mL of unprocessed urine, 8 mL of urine processed by ultracentrifugation, or 8 mL of urine concentrated to 1 mL using 100K centrifugal filter tube and processed using the EasySep™ Human Pan-Extracellular Vesicle Positive Selection Kit without or with the pre-clearing step.

NOTE: Aquaporin 2 is a renal collecting duct marker representative of renal epithelial-derived cargo from isolated EVs.

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