miniPURE-EVs-Spin

Centrifugal Spin Columns for extracellular vesicles and nanoparticle isolation

Product Code: HBM-mPEVS-##. Quantity: 12 or 24 spin columns

HansaBioMed Life Sciences

Pioneering the EV Field

About miniPURE-EVs-Spin Columns.

Size Exclusion Chromatography (SEC) is a very efficient method for separating EVs from the circulating proteins not affecting the original shape and functionality of the vesicles.

miniPURE-EVs-Spin is a SEC spin column designed for isolating EVs in a fast and easy way from small volume amounts of different fluids. Additionally, the column can be used for removal of small molecules from purified EVs, as the eccess of a dye after EV labeling procedure.

miniPURE-EVs Spin column is suitable for EV isolation form multiple fluids, including cell conditioned media, plasma, serum, urine. Moreover, the column is an optimal platform for the removal of unbound dye post particle labelling, removing traces of both membrane dyes or fluorophore conjugated antibodies.

VOLUME PROCESSABLE: 20 – 200 µl (0.02 – 0.2 ml)

1. Procedure for EV Isolation.

1. Sample preparation.

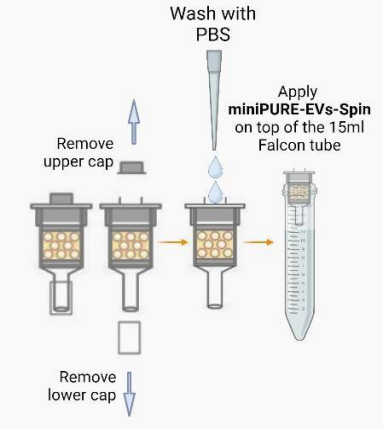
Prepare the sample by centrifugation steps as suggested in the table below:

Fluid	Suggested	Optional
Plasma	10 min at 300 g (save super) 20 min at 1200 g (save super)	30 min at 10000 g (to eliminate particles > 200 nm)
Serum	10 min at 300 g (save super) 20 min at 1200 g (save super)	30 min at 10000 g (to eliminate particleses > 200 nm)
Urine	10 min at 300 g (save super)*	
Cell media	10 min at 300 g (save super) 20 min at 1200 g (save super)*	30 min at 10000 g (to eliminate particles > 200 nm)

* Diluted fluids as Urine or Culture Media may require concentration prior EV isolation by the spin column, in case of poor yield. Concentration can be performed with EV-Spinner (100K) or Amicon filters (10K or 100K)

1.2 Column preparation.

- miniPURE-EVs-Spin columns are provided with a layer of preservative buffer.
- Leave the column at RT for 30 minutes before the use.
- Open both upper and lower cap of the column, place column in 15mL Falcon tube and centrifuge at 200xg for 3 min (do not close the lid of falcon tube during centrifugation).
- Wash the column with 3 volumes of PBS 1x buffer (3 x 400 µl) by adding buffer and repeating centrifugation 200xg, 4 min to eliminate preservative buffer residues



1.3 Sample loading and EV Isolation.

- Load thecolumn with sample containing EVs. Place column on top of a new Falcon tube and centrifuge at 200xg for 3 min. Collect the eluted Evs in the falcon tube.

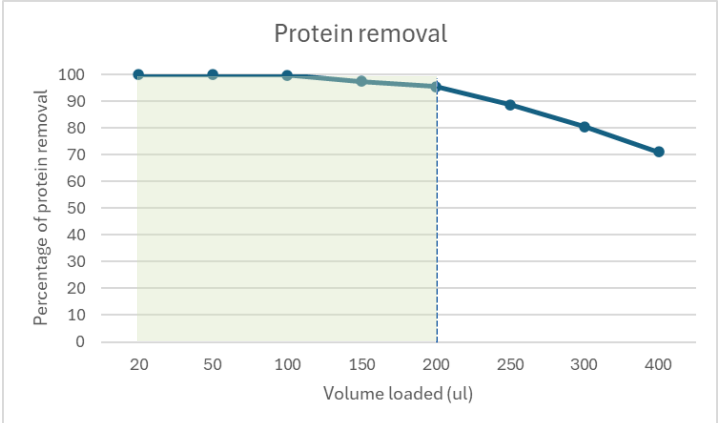
1.4 Column washing.

- Regular washing (EV purification and removal of antibody labelling): wash the column from the residues of sample with 0.4 ml of sodium hydroxide 0.5 N and spinning at 200xg for 3 minutes. Perform 3 additional washes with PBS 1x (3 x 400 µl) and repeat the centrifugation at 200xg for 4 min. After the last washing step add to the column 0.4 ml of PBS 1x and close both upper and lower caps. The column can be stored at 4 – 8°C and it is reusable 3 times.

- Membrane lypophilic dyes: Removing lypophilic dye residues can be difficult. After the use of lypophilic dyes the column should be washed 3 times with sodium hydroxide 0.5 N. If the column appears clean perform 3 additional washing steps with PBS 1x, then proceed as described above (1.4). If the color of lypophilic dye persists in the matrix gel the column should be disposed.

2. Column performance.

miniPURE-EVs-Spin column can remove the 99% of contaminant protein from EVs, processing from 20 – 100 µl per column and over the 95% of proteins processing 150 – 200 µl. For a highly pure particle yield it is not recommendable to load over 200 µl of sample in the column.



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3. Implementation of particle recovery

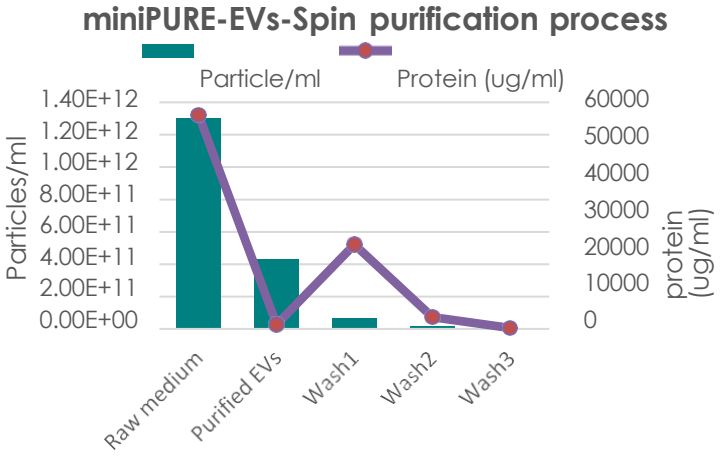
For sample loading 20 – 100 µl only. The recovery of particles can be implemented performing a second elution step. Following the regular procedure described at 1.3, the column can be loaded with 50 µl of PBS 1x, and the spinning procedure can be repeted (200xg, 3 min). The new eluted fraction (approximately 50 µl) can be added to the fraction collected in the step 1.3. Example of purification process is described in paragraph 4.

4. Example of column application.

4.1: EV purification from biofluids

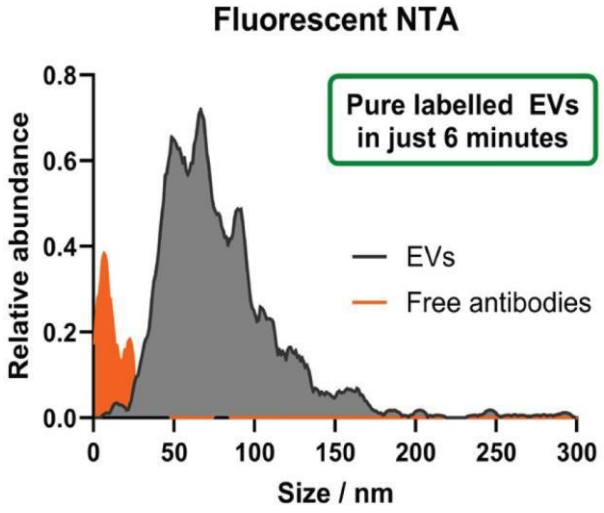
miniPURE-EVs-Spin column was filled with 100 µl of raw medium. EVs were eluted following 2 step centrifugation at 200xg. 3min.

First step was 100 µl sample, second step was 50 µl PBS, total elution was done in 150 µl (turnaround time approximately 6 min). Each filtrate after washing steps was also evaluated for particle number and protein amount.



4.2: EV labelling; removal of antibody excess.

COLO-derived EVs were incubated with anti-CD9 antibody (Alexa Fluor 488 conjugate) at 37°C, for 1.5h. After the incubation, the sample (100 µl) was loaded onto the miniPURE- EVs Spin column and centrifuged for 3 minutes at 200xg. Additional, 100 µl of PBS was loaded and centrifugation was repeated. Total eluate of 200 µl, containing pure labeled EVs.



4.3: EV labelling; removal of membrane dye excess.

U87-derived EVs were incubated with membrane dye at 37°C, for 1 hour. After the incubation, the sample (100 µL) was loaded onto the MiniPURE-EVs Spin column and centrifuged for 3 minutes at 200xg. The total eluate of 100 µL, containing pure labeled EVs, was collected and analyzed with NTA in both scatter and fluorescence modes. A comparative study was performed in parallel using a competitor's spin column.