

PURE-EVs: Size Exclusion Chromatography columns for Exosome and Microvesicle isolation.

PURE-EVs.

Cat Code: HBM-PEV-##. Quantity: 5 or 10 SEC columns

PURE-EVs Columns.

Size Exclusion Chromatography (SEC) is considered one of the best methods for isolating and purifying exosomes and extracellular vesicles (EVs) from different matrices. In particular, this technique is very efficient for separating EVs from the circulating proteins and does not affect the original shape and functionality of the vesicles. PURE-EV is a SEC column designed for isolating EVs in a fast and easy way from the volume amounts suggested:

Fluid	Volume amount
Plasma	0.5 ml up to 2 ml
Serum	0.5 ml up to 2 ml
Urine	0.5 ml up to 2 ml (concentrated 10 fold)
Cell media	0.5 ml up to 2 ml (concentrated 10 fold)

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 vesicles > 200 nm)
Serum	10 min at 300 g (save super) 20 min at 1200 g (save super)	30 min at 10000 g (to eliminate vesicles > 200 nm)
Urine	10 min at 300 g (save super) Concentrate 10 fold in MWCO concentrator.	
Cell media	10 min at 300 g (save super) 20 min at 1200 g (save super) Concentrate 10 fold in MWCO concentrator	Centrifuge before MWCO concentration, 30 min at 10000 g (to eliminate vesicles > 200 nm).

* Other biofluids which present a diluted population of EV can be concentrated 10 fold in MWCO concentrators. Viscous fluids (saliva) must be diluted in PBS 1x before to proceed to the EV isolation in SEC columns.

2- EV isolation.

- Open the upper cap of the PURE-EVs columns and rinse the column with 500 µl up to 2 ml of sample containing EVs (Fig 1).
- Open the lower cap. Fluid starts to flow through the column (Fig 1).
- Collect 500 µl fractions.

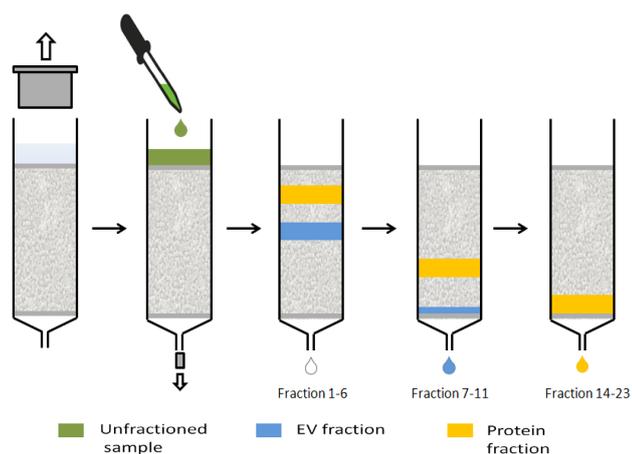


Fig1

Results and EV separation.

PURE-EVs column was rinsed with 1 ml of human plasma, 24 fractions (500 µl each one) have been collected and analyzed by ELISA ExoTEST™ assay and by BCA test for determining respectively vesicle and total protein content. EVs are eluted in fractions 6 - 11 (turnaround time approximately 15 min), whereas plasma circulating proteins corresponded to the fractions 14 - 24 (Fig 2).

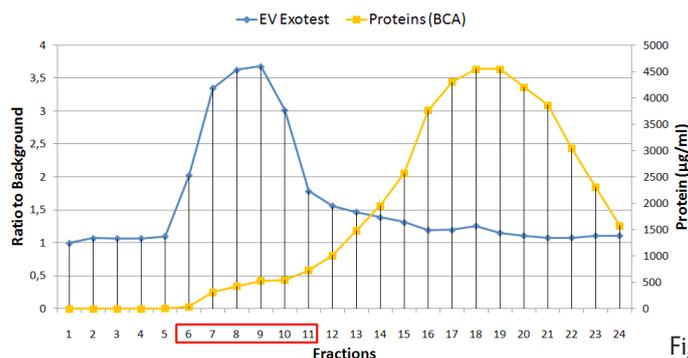


Fig2

EV isolation: FAST protocol.

The FAST protocol allows to obtain EV preparation in approximately 15 minutes, without collecting all the 500 µl fractions.

- Prepare the samples as indicated in "Sample preparation" paragraph.
- Open the upper cap of the PURE-EVs columns and rinse the column with 500 µl up to 2 ml of sample containing EVs.
- Open the lower cap. Fluid starts to flow through the column (Fig 3).
- Collect and discard the first 3 ml fraction, which does not contain vesicles.
- Collect and save the second 2 ml fraction, containing EVs (Fig 3).

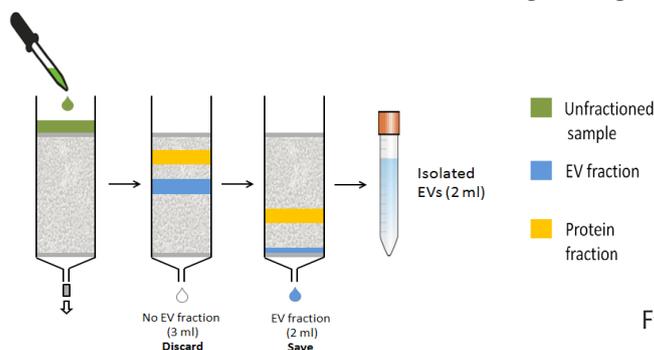
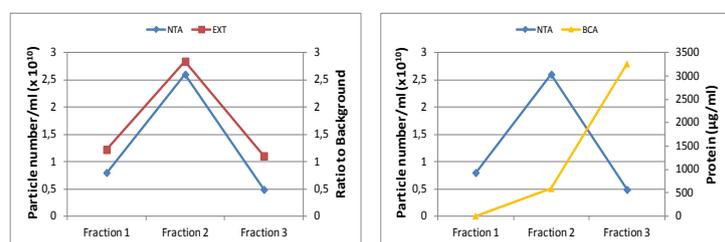


Fig 3

EV separation with FAST protocol.

Collected fractions were analyzed by NTA (Nanosight LM10), ExoTEST and by BCA assay for determining EV and total protein content (Fig 4). EVs are eluted in fraction 2.



EV elution peak. ExoTEST™ vs NTA analysis.

EV elution vs circulating protein elution. NTA analysis compared to protein BCA test.

Fig 4

Related product.

Cat. Code	Volume	Package
miniPURE-EV: Size Exclusion Chromatography columns		
HBM-mPEV-##	500 µl - 100 µl	10 or 20 Columns
PURE-EV PLUS: Size Exclusion Chromatography column and MWCO concentrator		
HBM-PEV-##	1.5 ml - 500 µl	5 or 10 Columns + 5 or 10 MWCO concentrators
miniPURE-EV PLUS: Size Exclusion Chromatography column and MWCO concentrator		
HBM-mPEV-##	500 µl - 100 µl	10 or 20 Columns + 10 MWCO concentrators

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