EV MiniSEC Columns: Size Exclusion Chromatography columns for Exosome and Microvesicle isolation.

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EV MiniSEC Columns (NBP3-41043)

Quantity: 10 or 20 SEC columns

EV MiniSEC 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.
EV MiniSEC is a SEC column designed for isolating EVs in a fast and easy way form small volume amount 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.

Fluid	Volume amount
Plasma	100 µl up to 500 µl
Serum	100 µl up to 500 µl
Cell medium	100 µl up to 500 µl, pre-concentrated 10 folds.
Urine	100 µl up to 500 µl, pre-concentrated 10 folds.
Other samples	100 µl up to 500 µl

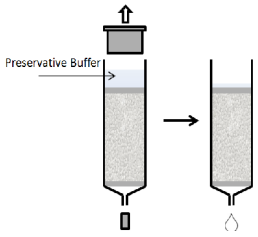
Procedure for EV isolation.

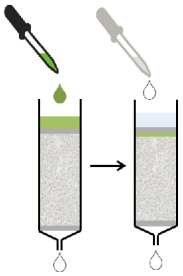
1. Sample preparation.
Prepare the sample by centrifugation steps as suggested in the table below:

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 folds*.	
Cell media	10 min at 300 g (save super) 20 min at 1200 g (save super) Concentrate 10 folds*.	

* It is recommended the use of Tangential Flow Filtration (TFF)-EV Concentrator (NBP3-11762) for concentrating the diluted fluids.
Alternatilvely, MWCO concentrators (100K) can be used.

2. Column preparation.
- EV MiniSEC Columns are provided with a layer of preservative buffer.
- Open the upper and the lower cap of the EVs column and let to flow almost all the buffer throught the column, avoiding to dry the surface of the gel.
- Wash the column with 3 volumes of PBS 1x buffer (3 x 4 ml) to eliminate preservative buffer residues.



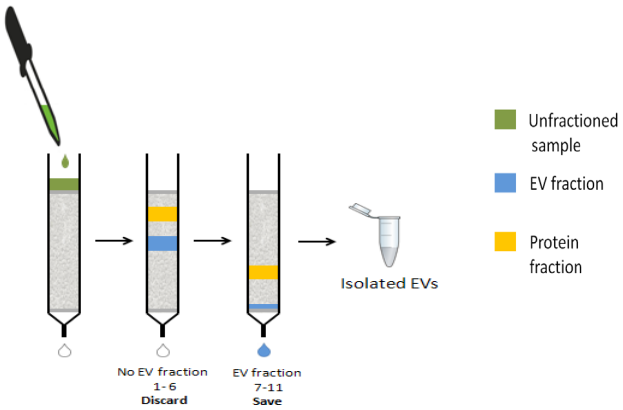


3- Sample loading.
- Rinse the column with 100 µl up to 500 µl of sample containing EVs.
- Collect 100 µl fractions.
- When the sample is inside the gel matrix rinse the column with PBS 1x.
PBS 1x is the mobile phase of SEC column, do not let the column to get dried.

4- EV isolation.
Separation of EV and circulating proteins proceeds as indicated in the figure 1 (collecting 100 µl fractions).

5 Column washing.
- After all fractions are collected wash the column from the residues of sample with approximately 10 ml of PBS. Never get the column dried. After the last washing step add to the column 0.5 ml of PBS 1x and close the caps.

Column can be stored at 4°C and reused up to 5 times.



EV separation.

EV MiniSEC Column was filled with 500 uL of medium from HCT116 cells, previously concentrated by TFF-Easy. 20 fractions (100 µl each one) have been collected and analyzed by ELISA assay (CD81 marker) and by BCA test for determining respectively vesicle and total protein content. EVs are eluted in fractions 9 - 14 (turnaround time approximately 10 min), whereas circulating proteins corresponded to the fractions 16 - 20.