ExoUltra: Size Exclusion Chromatography columns for extracellular vesicle isolation.

ExoUltra

Cat Code: K1246-5 and K1246-10.
Quantity: 5 or 10 SEC columns

ExoUltra Columns.

Size Exclusion Chromatography (SEC) is considered one of the best methods for isolating and purifying Extracellular Vesicles (EVs) from different matrices. This technique is very efficient for separating EVs from the circulating proteins and does not affect the original shape and functionality of the vesicles.
ExoUltra is a SEC column designed for isolating EVs in a fast and easy way form the volume amounts suggested:

Fluid	Volume amount
Plasma	0.2 ml up to 2 ml
Serum	0.2 ml up to 2 ml
Urine	0.2 ml up to 2 ml *
Cell media	0.2 ml up to 2 ml *
* For diluted fluids concentration 10 fold is recommended.	

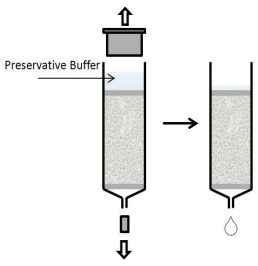
Procedure for EV isolation.

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

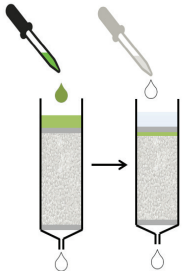
Fluid	Suggested	Optional
Plasma	10 min at 300 g (save super) 20 min at 1200 g (save super)	30 min at 10000 g (to eliminate vesicles > 200 nm)
Serum	10 min at 300 g (save super) 20 min at 1200 g (save super)	30 min at 10000 g (to eliminate vesicles > 200 nm)
Urine*	10 min at 300 g (save super).	
Cell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

* Biofluids which present a diluted population of EVs, can be concentrated 10 folds. Viscous fluids (saliva) must be diluted in PBS 1x before to proceed to the EV isolation in SEC columns. Use of TFF-Simple for concentrating the diluted matrices is recommended. Alternatively MWCO concentrators (100K) can be used.

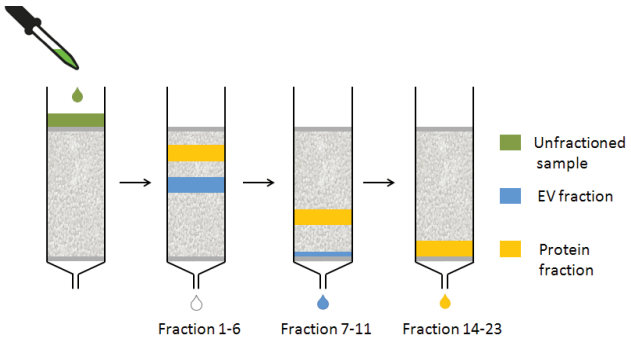
2- Column preparation.
- ExoUltra columns are provided with a layer of preservative buffer.
- Open the upper and the lower cap of the ExoUltra column and let to flow almost all the buffer through the column, avoiding to dry the surface of the gel.
- Wash the column with 3 volumes of PBS 1x buffer (3 x 10 ml) to eliminate preservative buffer residues.



3- Sample loading.
- Rinse the column with 200 µl up to 2 ml of sample containing EVs.
- Collect 500 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.



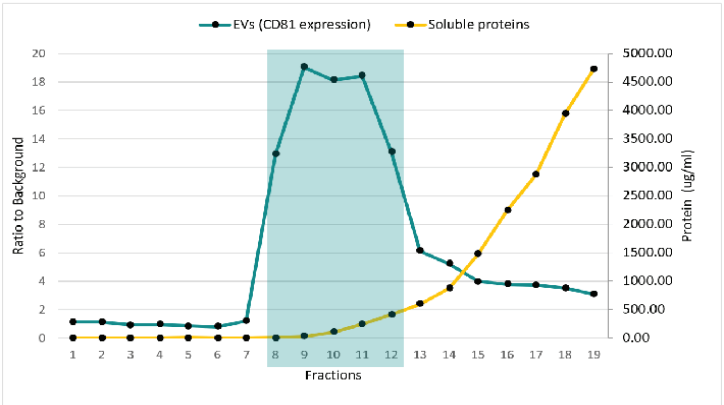
4- EV isolation
Separation of EV and circlating proteins proceeds as indicated in the figure below (collecting 500 µl fractions).



5- Column washing.
- After all fractions are collected wash the column from the residues of sample with approximately 20 ml of PBS. Never get the column dried. After the last washing step add to the column 1 ml of PBS 1x and close the caps. Column can be stored at 4°C and reused up to 5 times.

Results and EV separation.

ExoUltra column was filled with 2 ml of medium from HCT116 cells, concentrated 10 folds by TFF-Simple. 24 fractions (500 µl each one) havebeen collected and analyzed by ELISA ExoMeasure assay (CD81 marker) and by BCA test for determining respectively vesicle and total protein content. EVs are eluted in fractions 8 - 12 (turnaround time approximately 15 min), whereas circulating proteins corresponded to the fractions 14 - 24.

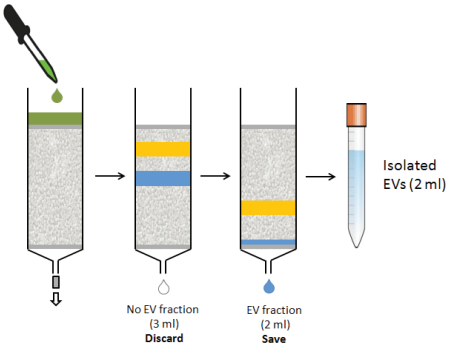


EV isolation: FAST protocol.

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.
- Prepare the column as indicated in "Column preparation" paragraph.
- Fill the column with 200 µl up to 2 ml of sample containing EVs.
- Collect and discard the first 3 ml fraction (void volume), which does not contain vesicles*.

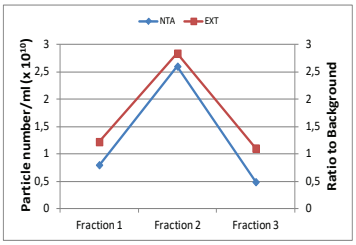
*When the sample is inside the gel matrix rinse the column with PBS 1x. PBS 1x is the mobile phase of SEC column, do not let the column to get dried.

- Collect and save the second 2 ml fraction, containing EVs.
-After EVs are collected wash the column as indicated in "Washing column paragraph". Store the column at 4°C.

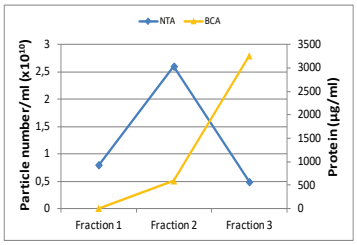


EV separation with FAST protocol.

Collected fractions were analyzed by NTA (Zetaview, Particle Metrix), ExoMeasure and by BCA assay for determining EV and total protein content. EVs are eluted in fraction 2.



EV elution peak. ExoMeasure vs NTA analysis.



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

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