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

EV MaxiSEC Columns (NBP3-11764)

Quantity: 3 SEC columns

EV MaxiSEC Columns.

Size Exclusion Chromatography (SEC) is considered one of the best methods for isolating and purifying exosomes and extracellular vesicles (EVs) from different matrices. EV MaxiSEC allow the isolation of EVs from big volumes (up to 20 ml) and the column is particularly recommended for working with diluted matrices as urine or cell culture media.

Fluid	Volume amount
Urine	5 ml up to 20 ml (concentrated 10 fold)
Cell media	5 ml up to 20 ml (concentrated 10 fold)

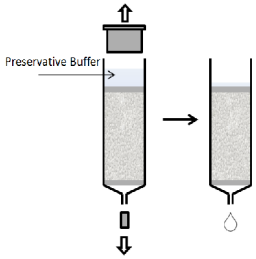
Procedure for EV isolation.

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

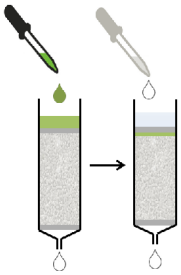
Fluid	Suggested	Optional
Urine	10 min at 300 g (save super). Concentrate 10 fold.	
Cell media	10 min at 300 g (save super). 20 min at 1200 g (save super). Concentrate 10 fold.	To eliminate big vesicle (> 200 nm): centrifuge 30 min at 10 000g before applying MWCO concentrator.

* Other biofluids which contain a diluted population of EVs can be concentrated 10 fold.

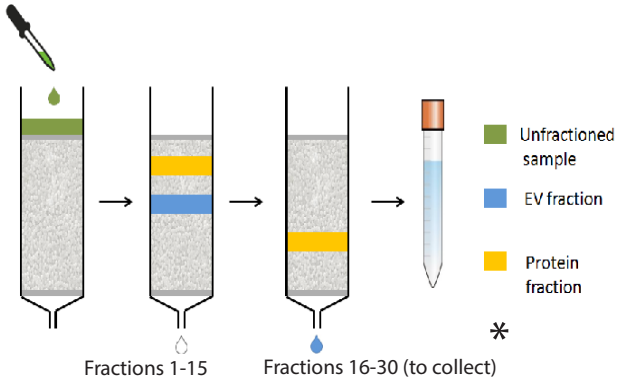
2- Column preparation.
- EV MaxiSEC columns are provided with a layer of preservative buffer.
- Open the upper and the lower cap of the PURE-EVs column and let to flow almost all the buffer throught the column, avoiding to dry the surface of the gel.
- Wash the column with 3 volumes of PBS 1x buffer (3 x 50 ml) to eliminate preservative buffer residues.



3- Sample loading.
- Load into the column 5 ml to 20 ml of your sample containing EVs.
- Collect 1 ml fractions.
- When the sample is inside the gel matrix, constantly add PBS 1x. PBS 1x is the mobile phase of SEC column, do not let the column get dried.



4- EV isolation.
Separation of EVs and circulating proteins proceeds as indicated in the figure below (the volume of fractions is 1 ml). The example is given for 20 ml of cell culture medium (10 fold priorly concentrated).



* The fractions containing EVs have to be determined experimentally by the users. Elution process indicated in the image above is for 20 ml sample volume. If the volume loaded into the column is smaller than 20 ml, then vesicles stop to come out in the earlier fractions.

5- Column washing.
- After all fractions are collected wash the column with approximately 150 ml of PBS to eliminate the residues of sample. Never get the column dried. After the last washing step add 10 ml of PBS 1x to the column and close the caps. Column must be stored at 4°C and can be reused up to 5 times.

Results and EV separation.

PURE-EVs column was loaded with 20 ml of cell medium10 fold concentrated in MWCO concentrator 100K. 40 fractions (1ml each) have been collected and analyzed by ELISA assay and by BCA test for determining respectively vesicle and total protein content. EVs are eluted in fractions 16 - 30 (turnaround time approximately 30 min), whereas plasma circulating proteins corresponded to the fractions 31 - 40.