ExoGenie Maxi Chromatography Columns

(Cat #BN00121 -3, -6, Maxi Size Exclusion Chromatography (SEC) Columns; Store at 4°C)

I. Introduction:

Exosomes are small endosome derived lipid nanoparticles (50-120 nm) actively secreted by exocytosis by most living cells. Exosomes have been isolated from diverse cell lines (hematopoietic cells, tumor lines, primary cultures, and virus infected cells) as well as from biological fluids in particular blood (e.g. serum and plasma from cancer patients) and other body fluids (broncho alveolar lavage fluid, pleural effusions, synovial fluid, urine, amniotic fluid, semen, saliva etc). Size Exclusion Chromatography (SEC) is considered one of the best methods for isolating and purifying exosomes and extracellular vesicles (EVs) from different matrices (urine and cell culture media). ExoGenie Maxi columns 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.

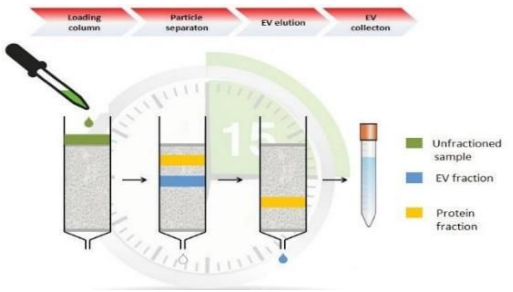


Figure 1. Exosome isolation from human plasma ExoGenie Maxi SEC Columns.

II. Application:

- Extracellular vesicles isolation from diluted matrices (biofluids and cell media).
- For EV purification of pre-isolated EVs from contaminants (5 ml up to 20 ml)
- Isolated exosomes are suitable for multiple analysis including NTA, ELISA, FACS, WB, EM, MS, nucleic acid extraction etc.

III. Key Features:

- Easy and fast protocol **(approximately 15 minutes)**
- Isolate exosome from diluted matrices and different sample types
- Easy to ship and store at 4°C

IV. Package Contents (for Exosome isolation from Biofluids or Cell Media):

Components	BN00121	BN00121	Part Number
Maxi SEC Columns	3	6	BN00121-1

V. User Supplied Reagents and Equipment:

- Single-use and/or pipettes with disposable tips 2-100 µl

VI. Shipment and Storage:

- Maxi SEC Columns can be shipped at room temperature or at 4°C but should be stored at 4°C for up to 12 months. DO NOT FREEZE!

VII. ExoGenie SEC Purification Protocol:

Fluid	Volume Amount
Urine	5 ml up to 20 ml (concentrated 10 fold)
Cell Media	5 ml up to 20 ml (concentrated 10 fold)

A. Sample Preparation:

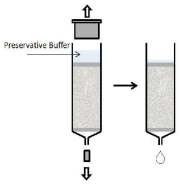
Prepare the sample by centrifugation steps as suggested in the table below

Fluid	Suggested	Optional
Urine	10 min at 300g (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.	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 in MWCO concentrators.*

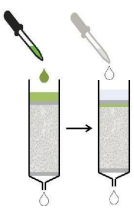
B. Column Preparation:

- ExoGenie Maxi 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 throughout the column, avoiding to dry the surface of the gel.



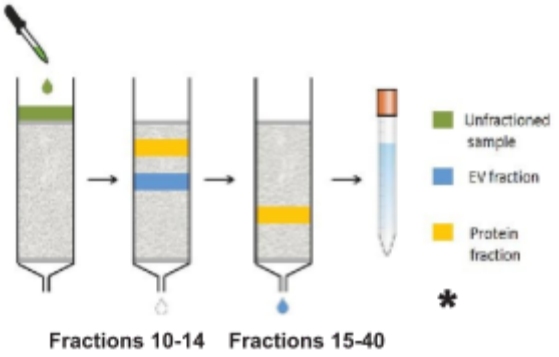
C. Sample Loading:

- 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.



D. EV isolation:

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



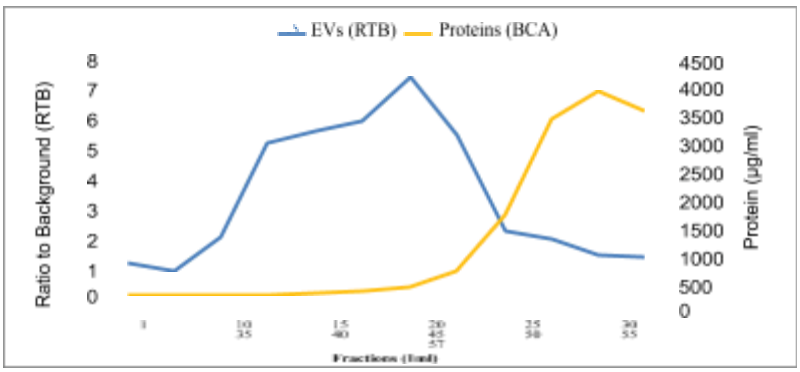
*The fractions containing EVs have to be determined experimentally by the users. Elution process indicated in the image above is for 20 ml sample volume. If the volume loaded into the column is smaller than 20 ml, then vesicles stop to come out in the earlier fractions.

D. 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 add10 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.

VIII. **Results and EV Separation:**

PURE-EVs column was loaded with 20 ml of cell medium10 fold concentrated in MWCO concentrator 100K. 60 fractions (1ml each) have been collected and analyzed by ELISA ExoGenie assay and by BCA test for determining respectively vesicle and total protein content. EVs are eluted in fractions 15 - 40 (turnaround time approximately 30 min), whereas plasma circulating proteins corresponded to the fractions 45 - 60.



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