Chromatography Columns (BN00117)

(Cat# BN00117 -5, -10, 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. This technique is very efficient for separating EVs from the circulating proteins without affecting the original shape and functionality of the vesicles. ExoGenie

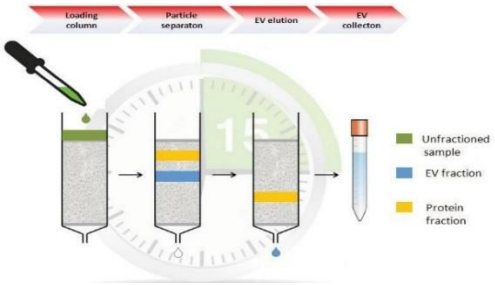


Figure 1. Exosome isolation from human plasma SEC Columns.

Chromatography Columns is a SEC column designed for isolating EVs in a fast and easy way.

II. Application:

- For the isolation of highly pure exosomes from biofluids (plasma, serum, urine) and cell media
- For EV purification of pre-isolated EVs from contaminants (500 µl up to 2 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 exosomes from different samples types
- Easy to store and ship at 4°C

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

Components	BN00117	BN00117	Part Number
SEC Columns	5	10	BN00117-1

V. User Supplied Reagents and Equipment:

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

VI. Shipment and Storage:

- 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. SEC Purification Protocol:

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 to 10-fold)
Cell Media	1.5 ml up to 2 ml (concentrated to 10-fold)

A. Sample Preparation:

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

Fluid	Suggested	Optional
Plasma	10 min at 300g (save super); 20 min at 1200g (save super)	30 min at 10000g (to eliminate vesicles > 200 nm)
Serum	10 min at 300g (save super); 20 min at 1200g (save super)	30 min at 10000g (to eliminate vesicles > 200 nm)
Urine	10 min at 300g (save super); Concentrate 10-fold using concentrators	
Cell Media	10 min at 300g (save super); 20 min at 1200g (save super) Concentrate 10-fold using concentrators	Centrifuge before using concentrators, 30 min at 10000g (to eliminate vesicles > 200 nm)

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

B. EV Isolation:

- Open the upper cap of the SEC 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 2). Collect 500 µl fractions

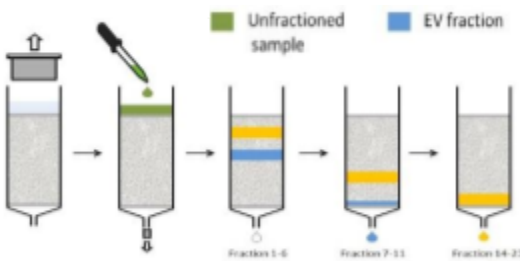


Figure 2. Exosome isolation from human plasma columns

C. Results and EV Separation:

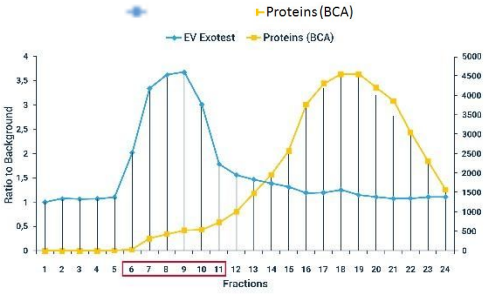


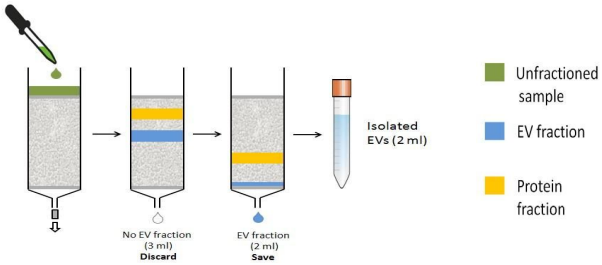
Figure 3. Matching of EV quantity and total protein content eluted in each single fraction. SEC Columns was rinsed with 1 ml of human plasma, 24 fractions (500 µl each one) have been collected and analyzed by ELISA assay and by BCA test for determining respectively vesicle and total protein content. EVs are eluted in fractions 6-11 (turnaround time approximately 15 min), whereas plasma circulating proteins corresponded to the fractions 14-24.

D. 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 ExoGenie SEC 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 4)
- Collect and discard the first 3 ml fraction, which does not contain vesicles
- Collect and save the second 2 ml fraction, containing EVs (Fig 4)

Figure 4.



E. EV Separation (FAST Protocol): Collected fractions were analyzed by NTA (Nanosight LM10), and by BCA assay for determining EV and total protein content (Fig 5). EVs are eluted in fraction 2.

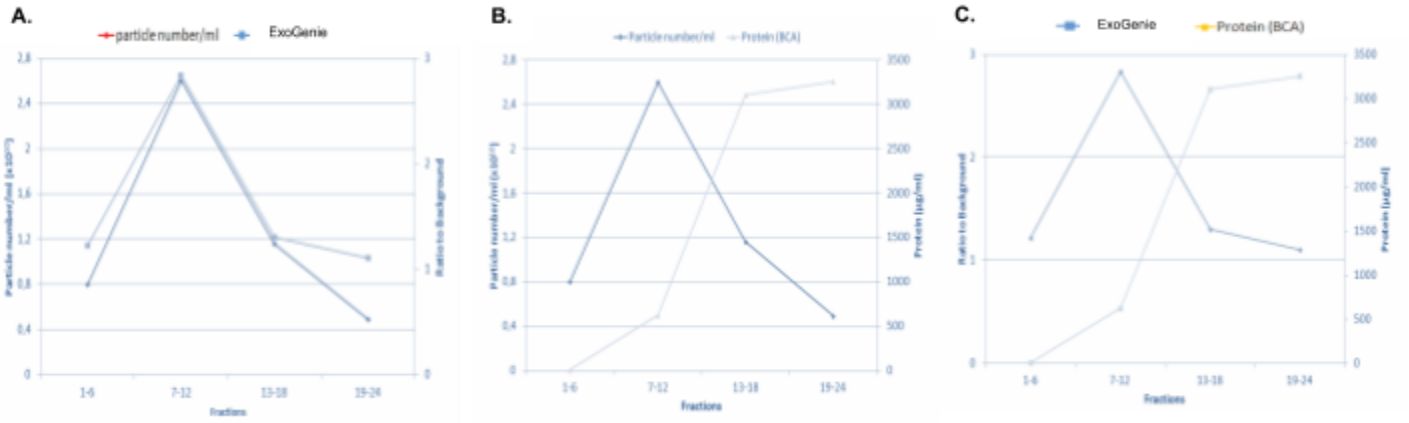


Figure 5. A. EV elution peak. ExoGenie vs NTA analysis. B. EV elution vs circulating protein elution. NTA analysis compared to protein BCA test. C. ExoGenie analysis compared to protein BCA test.

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