ExoGenie Mini Chromatography Columns (#BN00119)

(Cat# BN00119 -10, -20; Mini 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). Mini Size Exclusion Chromatography (Mini SEC) columns is considered one of the best methods for isolating and purifying exosomes and extracellular vesicles (EVs) from small volume amount from plasma and

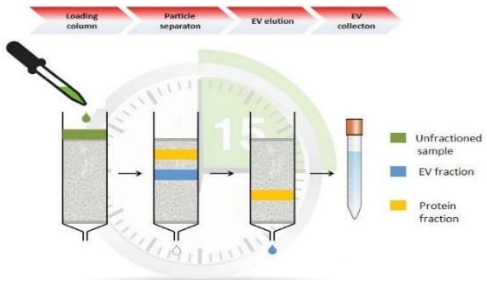

Figure 1. Exosome isolation from human plasma ExoGenie Mini SEC Columns.

serum or other biofluids. ExoGenie Mini 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 (100 µl up to 500 µl)
- 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 small volumes of sample and different sample types
- Easy to store and ship at 4°C

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

Components	BN00119	BN00119	Part Number
Mini SEC Columns	10	20	BN00119

V. User Supplied Reagents and Equipment:

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

VI. Shipment and Storage:

- Mini 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
Plasma	100 µl up to 500 µl
Serum	100 µl up to 500 µl

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)

B. EV Isolation:

- Open the upper cap of the ExoGenie Mini SEC Columns and rinse the column with 100 µl up to 500 µl of sample containing EVs.
- Open the lower cap. Fluid starts to flow through the column (Fig 2).
- Collect and discard the first 500 µl fraction not containing EVs.
- Collect and discard the second 500 µl fraction containing EVs.

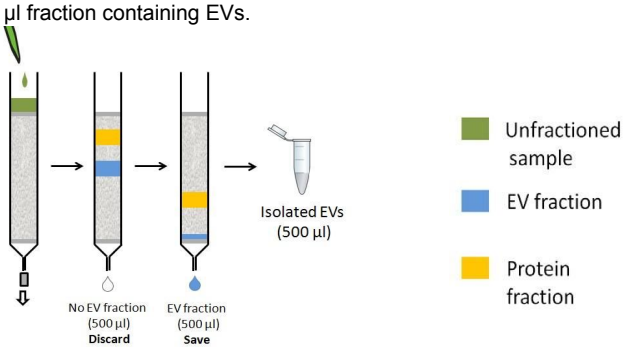

Figure 2. Exosome isolation from human plasma ExoGenie Mini SEC Columns.

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B. EV Separation: Collected fractions (500 µl) were analyzed by NTA (Nanosight LM10), ExoGenie and by BCA assay for determining EV and total protein content (Fig 3). EVs are eluted in fraction

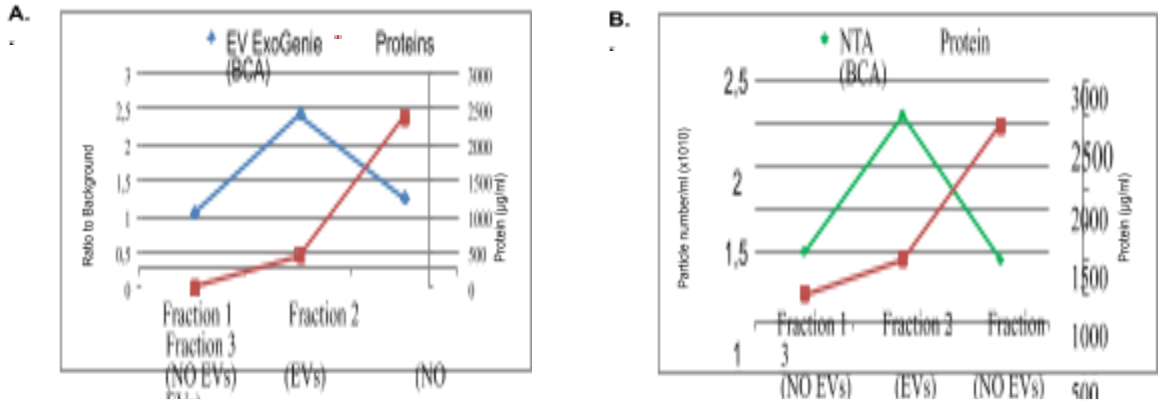
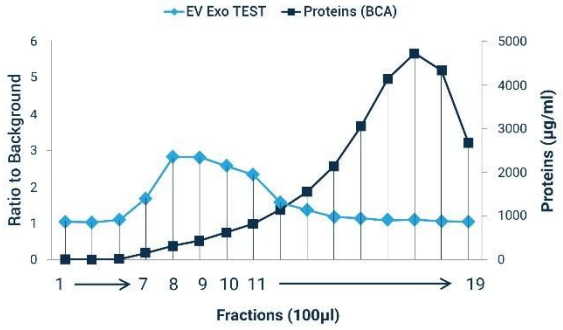


Figure 3. A. EV elution peak. ExoGenie vs NTA analysis. B. EV elution vs circulating protein elution. NTA analysis compared to protein BCA test.

PURE-EVs column was rinsed with 200 µl of human plasma, 19 fractions (100 µl each one) 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 7 - 11 (turn around time approximately 15 min), whereas plasma circulating proteins corresponded to the fractions 13 - 19 (Fig 4).



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