CELL guidance systems

Technical Note

High-throughput exosome isolation

Available in Canada from ...

MJS BioLynx INC.

1-888-593-5969 • www.biolynx.ca • tech@chromspec.com

High-throughput exosome isolation

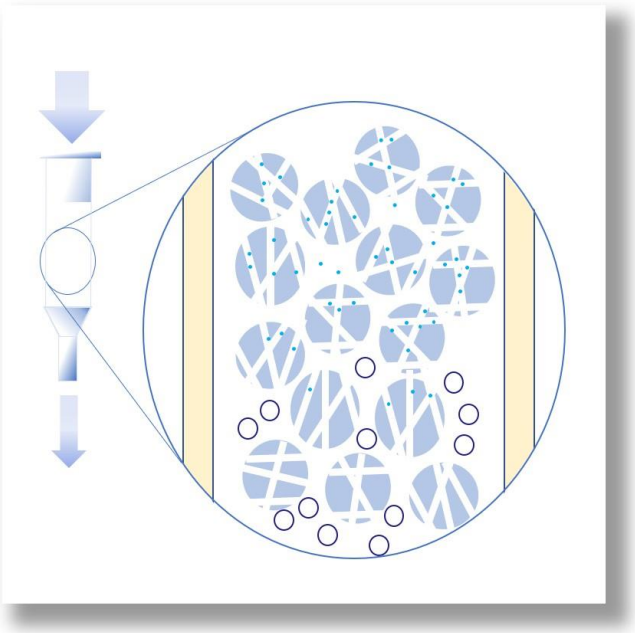
Exo-spin™ 96 (Cat EX07-96)

- **Experiment**
  96-well format exosome isolation
- **Exosome isolation time**
  96 samples in under 30 minutes
- **Initial sample volume**
  Up to 500 µl/well

Summary

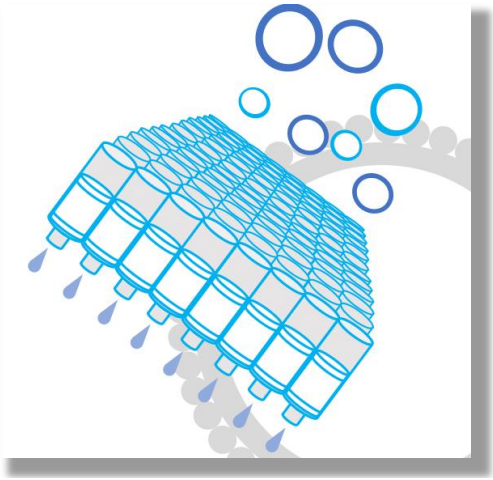
Size Exclusion Chromatography (SEC) separates particles in solution based on their size. SEC columns are packed with a resin containing porous spherical beads (see figure below). When a sample flows through the column matrix, the exosomes (open circles in the diagram below) run outside the beads and they elute first. Whereas, small particles and proteins (light blue dots) remain trapped in the pores of the beads and take longer to elute.

The Exo-spin™ range of columns offered by Cell Guidance Systems uses SEC to generate high quality purified extracellular vesicles (EVs), including exosomes, suitable for numerous research applications. The Exo-spin™ 96 plate is a high-throughput SEC column array, allowing up to 96 samples to be processed in parallel in less than 30 minutes.



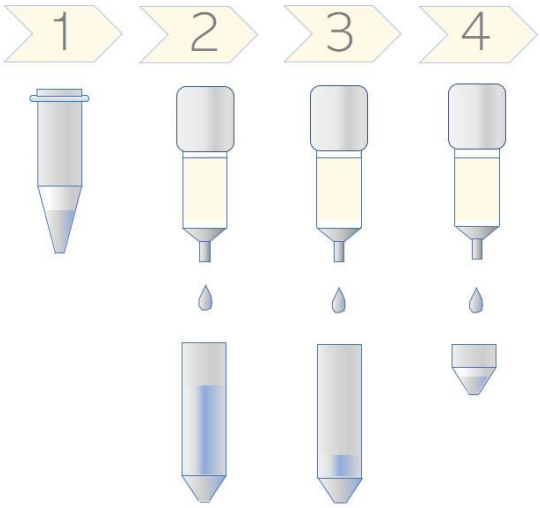
Key Features of Exo-spin™ 96

- **High-throughput** – Process 96 samples in parallel
- **Direct isolation** – Ideal for sera and plasma
- **High purity exosomes** – Eliminate over 97% of total protein
- **Fast** – Process 96 samples in under 30 minutes
- **Consistent** – Reliable yield throughout the plate
- **Gravity SEC** – Proven exosome isolation technology
- **Iterative loading** – Process up to 500 µl sample per column
- **Flexible use** – 12 detachable 8-column strips



Methods

Exo-spin™ 96 was used to isolate exosomes from FBS. The isolation was performed as briefly described below.



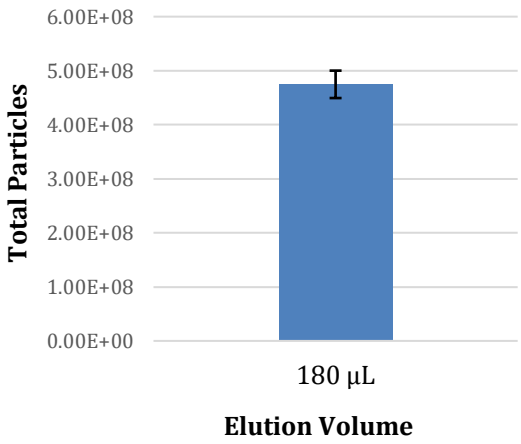
1. Two-stage clearing spin: the sample was centrifuged at 300 x g for 10 minutes. Then, the supernatant was transferred to a new centrifuge tube and centrifuged at 16,000 x g for 30 minutes to remove any remaining cell debris and large aggregates.
2. Equilibration of the columns with 2 x 250 µl PBS each.
3. 100 µl of sample was added to each column and allowed to enter. Flow-through was collected into the waste plate and discarded.
4. 180 µl of PBS was applied to each column and exosomes eluted into the collection plate.

The pre-cleared FBS was kept in low protein binding plastics and vortexed to ensure sample homogeneity prior to the addition to the columns. A multi-channel micropipette was used to add the sample to the SEC columns. The isolated exosomes collected in the 96-well collection plate were used for multiple downstream analyses.

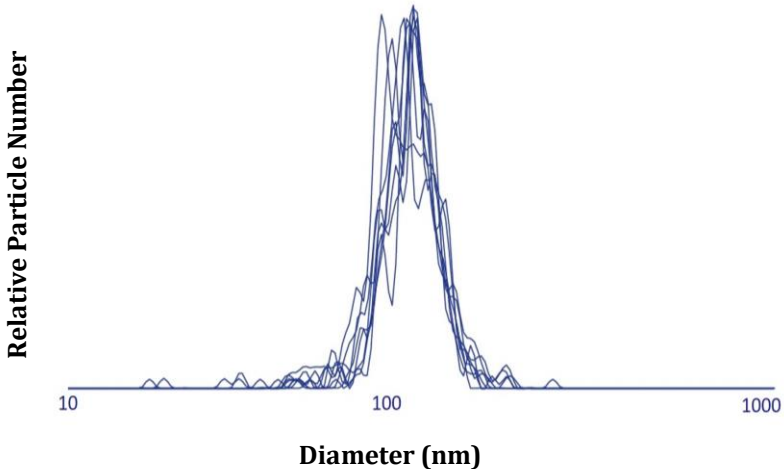
Results

Consistency

In order to check the yield and size profile consistency throughout the plate, the isolated exosome samples from a strip of 8 columns were diluted 1:100 in PBS and their numbers and size measured using NTA (ZetaView, Particle Matrix).



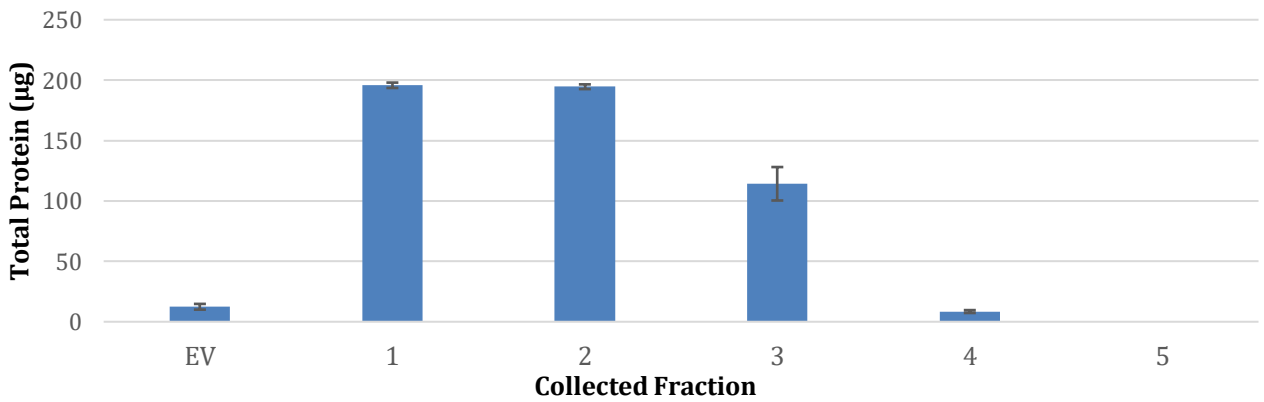
(**A**) This graph shows the average of the absolute numbers of particles detected by NTA. The variability, as illustrated by the error bar, is +/- 5% (standard deviation, n=8).



(**B**) This graph illustrates the size profile of each exosome isolation (n=8). There is a high degree of overlap between the peaks, demonstrating good consistency of the isolated exosome sizes.

Purity

After collecting the initial fraction containing the exosomes, a further 5 x 200 µl PBS was added to the column and each flow-through was collected. The protein content of all 6 fractions was measured using the Bradford assay.

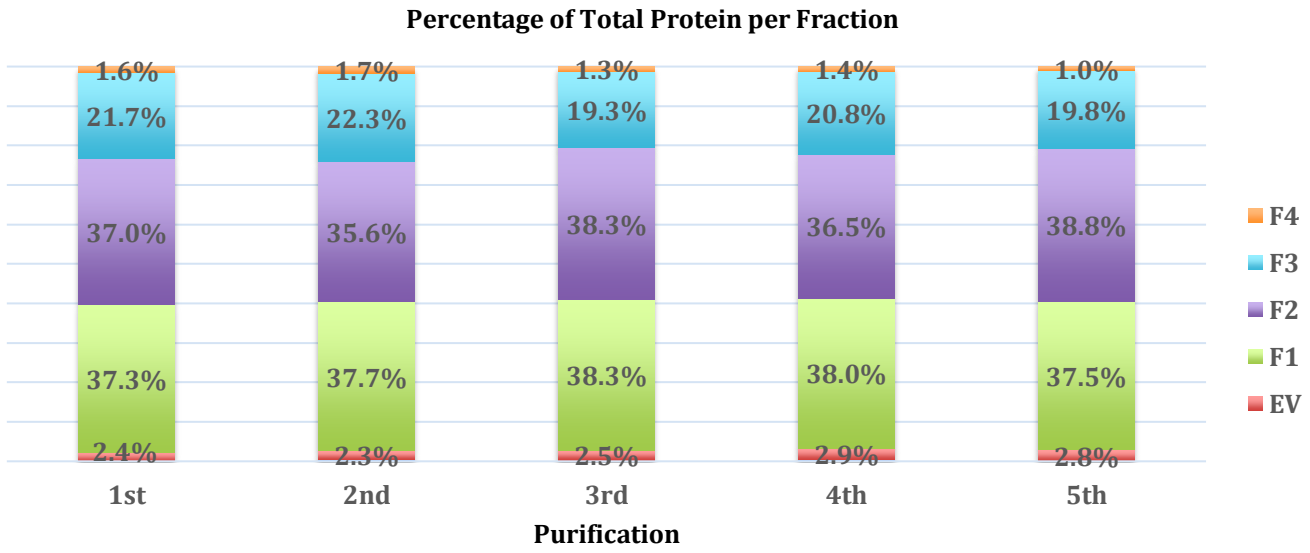


(**C**) The bar graph shows the total amount of protein found in the EV eluate as well as 5 subsequent fractions (n=8). The protein distribution demonstrates that over 97% of the total protein amount of the sample was separated off from the isolated exosomes (EV fraction).

Iterative loading

After eluting the first 180 µl EV-containing fraction, a further 4 x 200 µl PBS (800 µl total) was added to the columns. This removed all free protein from the previously loaded sample aliquot. A further 100 µl of sample was then added, isolated exosomes collected, and the flushing protocol repeated. In this manner, 5 x 100 µl of FBS were added to each column. In order to assess the purity and changes in protein elution, the total amount of protein in each fraction of the 5 iterative purifications was measured using the Bradford assay.

D



(**D**) As illustrated in the graph, the vast majority of the protein content is eluted in the three fractions after the EV eluate. The percentage of total protein eluted in the EV fraction is below 3%. (**NOTE: with human sera, the protein eluting with EVs is 1%).** This pattern of EV and protein elution stays consistent throughout the 5 sequential purifications (n=8), indicating that there is no carryover of protein from one cycle of purification to the next.

For more information on the Exo-spin™ size exclusion chromatography column range for exosome isolation, please visit our website www.cellgs.com.

Exo-spin™ mini

Exo-spin™ mini-HD

Exo-spin™ midi

Exo-spin™ 96

Cell Guidance Systems' reagents and services enable control, manipulation and monitoring of the cell, both *in vitro* and *in vivo*

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- Sustained Release

Exosomes
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- Detection
- NTA Service

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