Introducing the new Exo-spin™ mini-HD column
A reliable method for isolating high-quality exosomes

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Abstract

Exosome research plays an increasingly important role in therapeutics and diagnostics. Therefore, efficient and reliable methods for isolating exosomes are required.

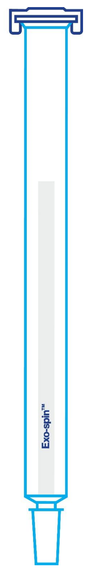
Among other methods, size exclusion chromatography (SEC) is widely used for exosome isolation from all biofluids, as it demonstrates highly pure and concentrated samples. Moreover, SEC can be performed under different protocols, including centrifugation and gravity flow. Under gravity, one sample can be collected into fractions and analysed for a complete high-resolution fractionation profile.

In this poster, we highlight the Exo-spin™ mini-HD kit as a novel tool to isolate exosomes from human serum as an example.

Exo-spin™ technology

Exo-spin™ technology combines **precipitation and SEC,** making it superior to techniques that rely solely on precipitation which result in co-purification of large amounts of non-exosomal proteins and other material, as well as carryover of the precipitant.

1. Remove cells and cellular debris by centrifugation
2. Precipitate exosome containing fraction (not required for blood sample (plasma and sera))
3. Add the exosome-containing pellet to the Exo-spin™ mini-HD column for SEC purification



SEC method

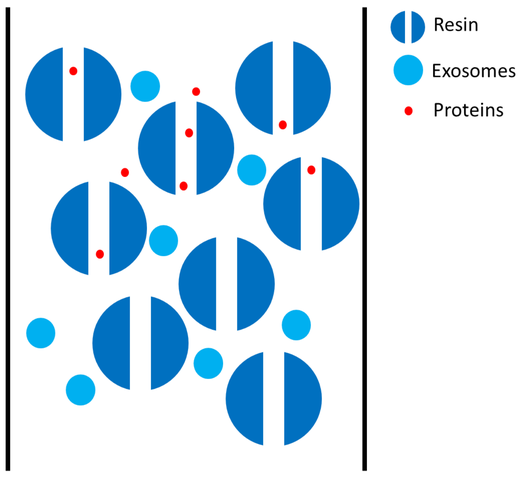


Figure 1: Introduction to SEC.
SEC is a method which separates particles in solution based on their size. The chromatography column is packed with stable polymeric beads (resin), creating a porous matrix. When the solution containing exosomes is added to the SEC column, the smaller particles will be trapped in the pores, while larger particles do not enter the pores and elute first. As a result, different elution fractions will contain particles of different sizes; first the large particles, followed by the smaller particles.

This technique is ideal to achieve highly purified samples in which exosomes are separated from other non-exosomal components, and it is compatible with both low and high initial sample volumes.

Exosomes isolation from human serum

Methods

10 ml of human serum starting volume was obtained from Cambridge Bioscience (UK).

Subsequently, 100 µl of this sample was added to the Exo-spin™ mini-HD column, followed by 100 µl of PBS then 23 x 200 µl PBS, until 24 fractions of 200 µl were collected. Consequently, the resulting fractions were analysed and a fractionation profile generated.

Results

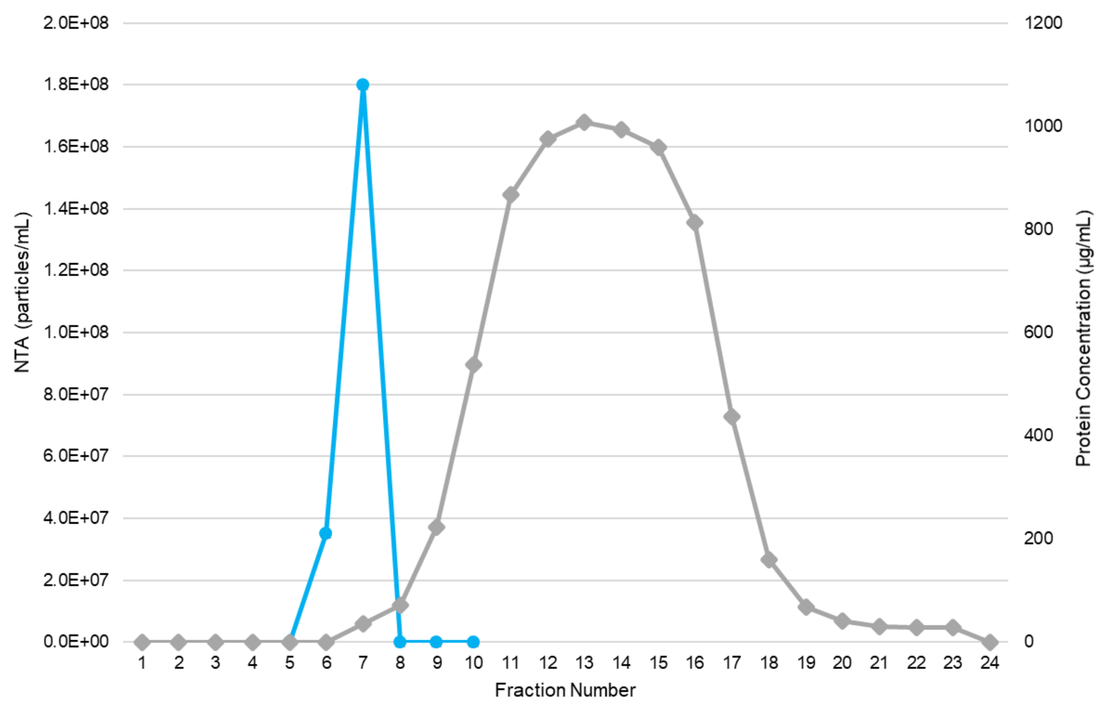


Figure 2: Human serum fractionation profile using Exo-spin™ mini-HD.
The protein concentration was estimated by Bradford assay (grey) and the particle concentration (blue) was measured by Nanoparticle Tracking Analysis (NTA) using ZetaView® instrument from Particle Metrix.

Fractionation analysis
From fractions 1 to 5, no exosomes were eluted from the Exo-spin™ mini-HD column. Fractions 6 and 7 contain the exosomes, with peak exosome concentration in fraction 7, and ultra-low protein contamination in both of these fractions. The vast majority of proteins present in the sample elute in the subsequent fractions.

Recovery
The exosome concentration of the human serum sample was performed prior to the isolation and 80% recovery was measured.

Conclusion
The Exo-spin™ mini-HD allows a high-resolution fractionation protocol for starting sample volumes from 1 to 75 ml (cell culture media, urine, saliva, CSF, and any other low-protein biofluids) and from 1 to 150 µl blood sample (plasma and serum). As the Exo-spin™ mini-HD operates by gravity, no special equipment is required.

Conclusions

- High-resolution protocol to optimize yield and purity
- Robust, simple, quick and economical
- SEC method for excellent exosome recovery

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