Frequently Asked Questions (FAQs)

Exo-spin™
Exosome Purification Kit

Version 1.0
Exo-spin™ Exosome Purification Kits

FAQs

• Which method is used in the Exo-spin™ kit to isolate exosomes?

The Exo-spin™ technology combines precipitation and size exclusion chromatography (SEC) columns.

• What is size exclusion chromatography (SEC)?

SEC is a method to separate particles in solution based on their size. The chromatography column is packed with stable polymeric beads, 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 molecules do not enter the pores and elute first. As a result, different elution fractions will contain molecules of different sizes; first the large particles, followed by the smaller particles.

• What is the precipitation method?

The precipitation method allows for the separation of solid substance from a given sample. Exo-spin™ technology uses this method to concentrate the exosomes prior to isolation with SEC columns.

• What is the Exo-spin™ buffer formulation?

The Exo-spin™ buffer formulation is a proprietary, polymer-based solution.

• What is the resin pore size of the Exo-spin™ columns?

The resin pore size of our Exo-spin™ columns is approximately 30 nm.

• Are Exo-spin™ columns ready-to-use?

All our Exo-spin™ columns are shipped pre-packed and equilibrated ready-to-use.

• Can the Exo-spin™ columns be re-used?

We do not recommend the re-use of Exo-spin™ columns.
• **What is the maximal limit of exosome-containing pellet to load into the Exo-spin™ columns?**

The maximal limit of exosome-containing pellet after the precipitation step will depend on the kit:
- EX01: Isolate exosomes from cell culture media, urine, saliva, and other low-protein biological fluids. Maximum volume: 50 ml
- EX02: Isolate exosomes from blood samples (sera and plasma). Maximum volume: 500 µl sera and 250 µl plasma

• **When purchasing Exo-spin™ kit, are collection tubes and PBS included?**

EX01 and EX02 kits are shipped with collection tubes and PBS (modified without calcium chloride and magnesium chloride). EX03, EX04 and EX05 contains only the SEC columns.

• **What incubation time is recommended for the precipitation step?**

For all biofluid type (except blood), between 1 hour and overnight is sufficient. Blood samples requires an incubation time of at least 5 minutes.

• **I do not have a low-speed centrifuge (i.e. 50 x g). What can I do?**

The low-speed centrifuge is only required for EX01, EX02 and EX03. For these type of columns, it is important to spin at 50 x g as the resin can easily get compressed at even 100 x g. An example of a low-speed centrifuge is CappRondo microcentrifuge (Capp®, CR-68X). As an alternative, the Exo-spin™ mini-HD (cat. EX05) kit can be used, as the protocol will be performed under gravity and therefore no specific material is required.

• **I do not have a high-speed centrifuge (i.e. 16,000 x g). What can I do?**

We do not recommend centrifuging at higher speeds, as this will very likely compromise the functionality of the column. However, you can increase the time of centrifugation by calculating the ratio of the recommended speed to the speed of your centrifuge. For example, if the protocol recommends centrifuging at 16,000 x g for 30 minutes, for a centrifuge with a maximum speed of 9,500 x g: 16000/9500=1.68 and 1.68*30 mins = 50.4 minutes.

• **After spinning the Exo-spin™ column, I can see that the column has changed color. Is it normal?**

The color retained in the column is due to proteins or small molecules which get trapped in the column, and therefore removed from the sample, improving the final eluate purity.

• **The final eluate is not transparent. Is it normal?**

Yes, the final eluate does not have to be transparent.

• **Can I use a smaller volume for the final eluate in order to concentrate the exosomes?**

We do not recommend using a smaller volume to elute. The protocol has been optimized to efficiently elute the exosomes in a 200 µl fraction. If the volume is lower, the eluate will contain mostly exosomes of a larger diametral size and therefore not offer whole representation of the
exosome population. If a higher concentration of exosomes in the final eluate is required, 100 kDa molecular weight cut-off (MWCO) filters can be used to concentrate the sample.

- How can I concentrate exosomes in cell culture medium?

Cell culture typically generates yields of $1 \times 10^9$ particles per ml on average. Use of cell culture devices such as the Integra CELLine flask can increase the concentration of exosomes in the media by around 10-fold, obviating the need for precipitation. When processing of larger sample volumes is required, precipitation buffer concentrates exosomes prior to column purification.

- After the precipitation step, my pellet is very large. Should I resuspend it in a higher volume than the recommended 100 µl?

The pellet can be resuspended in a higher volume, but no more than 100 µl of exosome resuspension should be applied to any one column, in order to avoid clogging. Therefore, if you do need to resuspend your pellet in a greater volume than 100 µl, the resuspension should be divided between multiple columns. Ideally, the volume loaded per column should be as close as possible to 100 µl. If the volume loaded is too high, the column may clog; if the volume is too low, exosomes will be retained on the column.

- After the precipitation step, my pellet is sticky. What should I do?

If your pellet is too sticky, resuspend it in more than 100 µl and subsequently use multiple columns per sample (no more than 100 µl per column) to improve the process.

- My sample does not elute from the column. What should I do?

Ensure that the outlet plug has been removed from the base of the column. The outlet plug must be removed before the screw cap. Furthermore, if the column has been centrifuged at excessive speed, it will be compromised and subsequently not function correctly. Be aware that some centrifuges cannot provide the low speed required.

- My sample contains a lower amount of exosomes than expected.

Ensure that the column does not dry out during the procedure. Any column that is spun for too long or at excessively high speed may dry out. If your column did not dry out, we recommend precipitating your sample with the Exo-spin™ buffer overnight instead of 1 hour. If you are working on cell culture medium, we recommend increasing cell conditioning time as well as seeding cells at higher density to facilitate secretion of more exosomes. Finally, adhere to the volumes indicated for sample addition to the column. If the sample volume is too small, the exosomes will be retained within the column.

- I am using mass spectrometry. Is the Exo-spin™ kit compatible?

Exo-spin™ kit EX02, EX03, EX04 and EX05 are compatible with mass spectrometry, depending on your sample and starting volume. Please refer to the user guides for more information.
• I would like to improve the signal of my exosome markers (CD9, CD63, or CD81). What should I do?

Validated exosomes antibodies are available from Cell Guidance Systems (catalogue codes EX201, EX202, EX203, and EX204). If you are interested in detecting the signal of extracellular vesicle (EV) surface markers, we recommend you use our TRIFic™ exosome detection assay.

• After isolation, how can I analyse the exosome size and concentration from my sample?

You can characterize your sample with nanoparticle tracking analysis (NTA). NTA is a technique used for the simultaneous measurement and analysis of the size and concentration of single nanoparticles with high precision, accuracy, and reproducibility. Cell Guidance Systems provide it as a service with ZetaView® machine from Particle Metrix GmbH (catalogue code ZV-1 and ZV-12). The NTA reports generated by Cell Guidance Systems provide complete detail on the diameter and size distribution of particles.

• How can I study exosome imaging, uptake or long-term tracking?

You can study exosome imaging, uptake or long-term tracking with our ExoFLARE™ exosome tracking assay (catalogue code EX301, EX302, and EX303). The ExoFLARE™ exosome tracking assay utilizes a combination of a FLARE (fluorescence activating response element) protein tag linked via a transmembrane domain to the individual tetraspanin proteins CD9, CD63, and CD81, together with a pro-fluorophore dye to allow tracking of exosomes.

• What is the recommended method for RNA extraction?

Any TRIzol-based method or equivalent can be used after Exo-spin™ isolation.
Cell Guidance Systems’ reagents and services enable control, manipulation and monitoring of the cell, both \textit{in vitro} and \textit{in vivo}.

**Growth Factors**
- Recombinant
- Sustained Release

**Exosomes**
- Purification
- Detection
- Tracking
- NTA Service

**Small Molecules**

**Cell Guidance Media**
- Pluripotent Stem Cells
- Photostable
- \textit{In Vitro} Blastocyst Culture
- ETS-embryo Culture
- Custom Manufacturing Service

**Matrix Proteins**

**Gene Knock-Up System**

**Cell Counting Reagent**

**Cytogenetics Analysis**

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