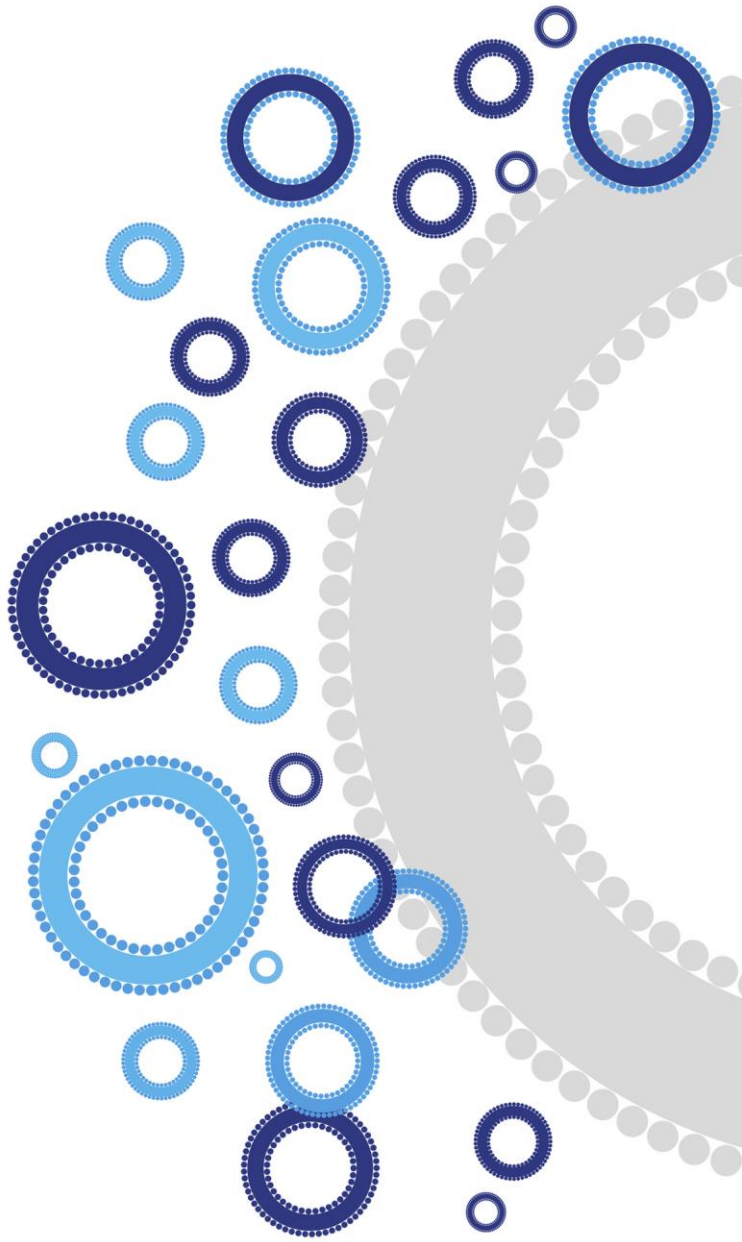
CELL guidance systems

Frequently Asked Questions (FAQs)



Exo-spin™

Exosome Purification Kit

Version 1.1

Exo-spin™ Exosome Purification Kits

FAQs

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

The Exo-spin™ kit technology combines precipitation and size exclusion chromatography (SEC) columns. SEC columns can also be purchased as a stand-alone product and used without prior precipitation of the sample.

- **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 substances 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 approximatively 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 reusing 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 selected, please refer to the individual user guides.

- **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 types (except blood), between 1 hour and overnight is sufficient. Blood samples require 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 Exo-spin™ kits. For these types of mini 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 (catalogue code EX05) kit can be used, as the protocol is performed under gravity and therefore no specific equipment is required.

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

We recommend to 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 minutes = 50.4 minutes.

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

Yes, this is normal. The color change of the column is due to proteins or small molecules which get trapped in the column.

- **The final eluate is not transparent. Is this 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 for EX01, EX02, and EX03 kits 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. For all Exo-spin™ kits, 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?**

Conditioned cell culture medium typically generates yields of $1x10^9$ 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, removing the need for precipitation. When processing of larger sample volumes is required, precipitation buffer concentrates exosomes prior to column purification.

- **EX01 and EX02 Exo-spin™ kits: 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 volume greater 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.
Please consider the use of multiple columns or select a different product such as Exo-spin™ mini-HD (catalogue codes EX05) or Exo-spin™ midi columns (catalogue codes EX04) for larger volumes.

- **For the EX02 Exo-spin™ kit, my pellet is particularly difficult to resuspend after the precipitation step. What can I do?**

Perform the next steps:

a) Centrifuge the pellet at 1,500 x *g* for 30 minutes, instead of 16,000 x *g* as instructed in step 5 of the EX02 user guide.
b) Aspirate supernatant.
c) Incubate the pellet for 10 minutes at 37°C.
d) Expel warmed PBS onto the pellet to break it up.
e) Resuspend the pellet using a pipette tip that has been cut to about 1/3rd of the way up to stop pellet blocking the pipette tip.

If still necessary, lower your starting volume to resuspend the pellet easier.

- **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 50 x *g* speed required for the EX01 and EX02 Exo-spin™ protocols.

- **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

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

- **After isolation, how can I analyze 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, using the ZetaView® machine from Particle Metrix GmbH (catalogue codes 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 codes 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 *in vitro* and *in vivo*

Growth Factors
- Recombinant
- Sustained Release

Exosomes
- Purification
- Detection
- Tracking
- NTA Service

Small Molecules

Cell Counting Reagent

Matrix Proteins

Cell Culture Media
- Pluripotent Stem Cells
- Photostable
- *In Vitro* Blastocyst Culture
- ETS-embryo Culture
- Custom Manufacturing Service

Gene Knock-Up System

Cytogenetics Analysis

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