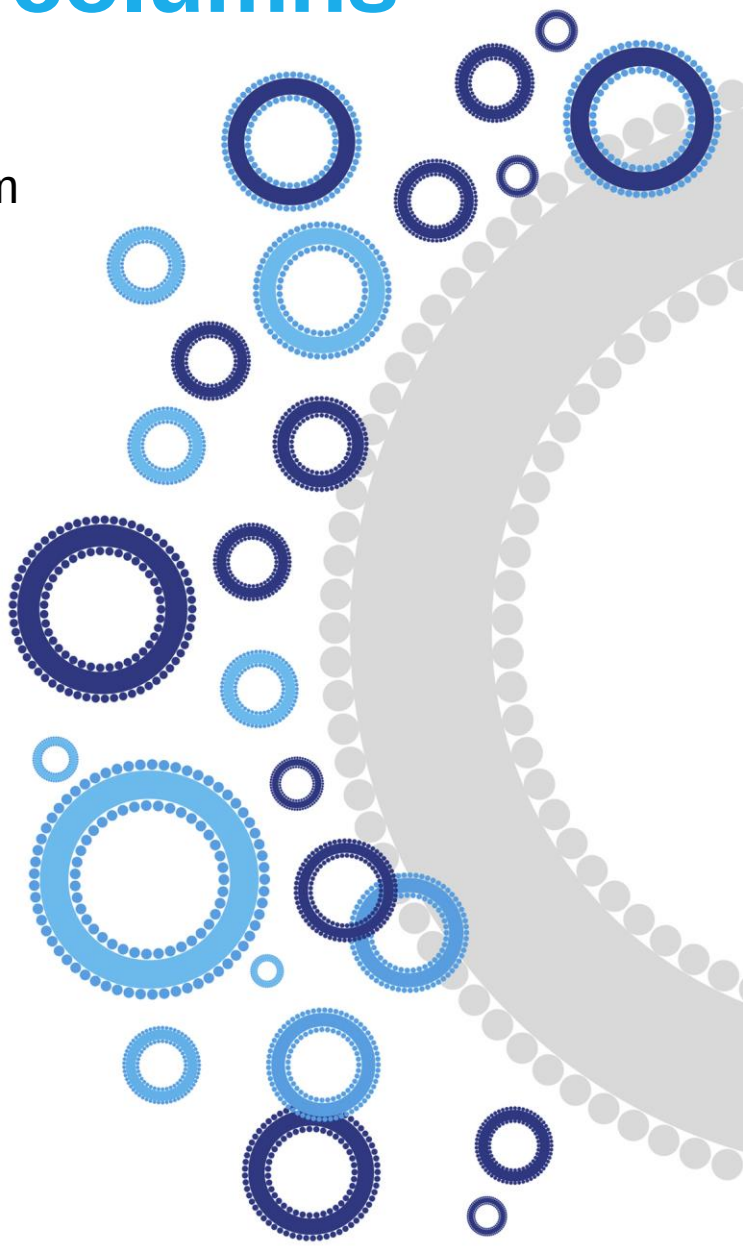
User Guide

Exo-spin™ mini columns

Exosome Purification Kit

For blood sera, cell culture medium
and labelled exosome samples

Cat EX03



Protocol Version 3.7

Contents

Exo-spin™ mini columns Exosome Purification Kit

Product components

EX03-8 Exo-spin™ mini columns kit (8 columns)

- 8 x Exo-spin™ mini columns with waste collection tubes
- 1 x User Guide

EX03-25 Exo-spin™ mini columns kit (24 columns)

- 24 x Exo-spin™ mini columns with waste collection tubes
- 1 x User Guide

EX03-50 Exo-spin™ mini columns kit (48 columns)

- 48 x Exo-spin™ mini columns with waste collection tubes
- 1 x User Guide

For all kits, large volume (15 ml or 50 ml) centrifuge tubes, PBS, and 1.5 ml microcentrifuge collection tubes are not supplied.

Storage

Upon receipt, store Exo-spin™ mini columns at 4°C.

Correctly stored components are stable for at least 3 months following purchase.

General exosome isolation information

A. Notes on blood samples collection

Sample collection and handling prior to purification may have a significant impact on the quality of purified exosomes (Witwer *et al*., 2013). Sera can contain many platelet-derived exosomes released after clot formation. Use of heparin-based anticoagulants is discouraged because of possible effects on downstream applications (e.g. PCR).

Platelet-derived exosomes may be released from platelets by the physical forces associated with the blood sampling procedure. Standardization of sampling site, needle gauge (wider may be better), and other variables is recommended. To ensure the sample is not contaminated by skin fibroblasts, it has been suggested that the first few milliliters of drawn blood should be discarded.

Collected blood should be handled gently and processed rapidly (within 30 minutes of drawing).

B. Proteomic analysis

Precipitants can interfere with mass spectrometry analysis and so precipitation should not be used when purifying exosomes if mass spectrometry is to be performed. In such cases, an alternative concentration method should be used instead of precipitation prior to using the Exo-spin™ columns.

Product information

Exo-spin™ technology combines Precipitation and Size Exclusion Chromatography (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 carry over of the precipitant. However, EX03 kit only contains SEC columns as the precipitation step is not required for some applications (e.g. mass spectrometry downstream application and/or ≤100 µl sera samples).

Isolated exosomes may be used in a variety of downstream applications including DNA and RNA studies, as well as in functional *in vitro* and *in vivo* exosome assays.

This kit has been developed to process ≤100 µl sera sample per column. Samples less than 100 µl in volume should be diluted with PBS to a final volume of 100 µl.

Exo-spin™ mini columns may be used to clean-up samples which have been purified by other methods, such as precipitation and ultracentrifugation, in order to remove proteins and smaller molecules. Exo-spin™ columns are also very effective for the clean-up of labelled exosome samples.

For more information on our exosome isolation range please refer to our website.

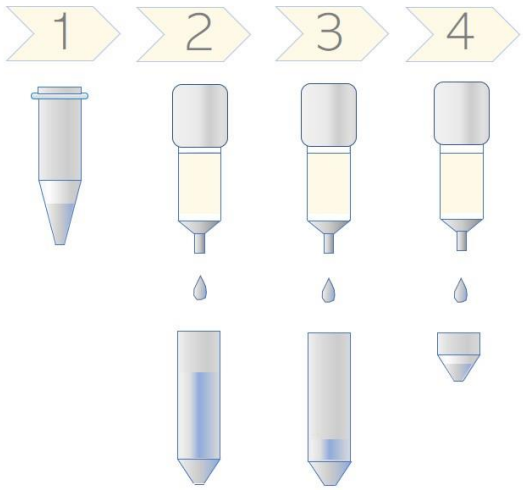
Protocol for purification of intact exosomes using Exo-spin™ mini columns

Supplied Exo-spin™ columns are pre-equilibrated with ultra-pure water containing 20% ethanol. The column matrix should be re-equilibrated with PBS prior to use.

A maximum sample volume of 100 µl sera may be used per column. For larger sample volumes, use multiple columns per sample.

For typical samples containing cells, such as sera, an additional step must firstly be performed to remove cells and cell debris, prior to purification of exosomes. **This step may be omitted for clean-up of secondary samples which do not contain cells.**

All centrifugation steps can be performed at room temperature or 4°C, unless otherwise specified.



Protocol overview.

A. Remove cells and cell debris

1. Transfer 100 µl of starting sample to a microcentrifuge (not supplied with kit) tube and spin at 300 *x g* for 10 minutes to remove cells.

2. Transfer supernatant to a new microcentrifuge tube and spin at 16,000 × *g* for 30 minutes to remove any remaining cell debris.

3. Transfer supernatant to a new microcentrifuge tube and add PBS if required so the sample has a total volume of 100 µl.

B. Exo-spin™ mini column preparation

4. Prepare the Exo-spin™ column prior to application of your sample.
   a. Equilibrate the column at room temperature for 15 minutes before use.

   b. **Remove the outlet plug before the screw cap** and place the Exo-spin™ column into the waste collection tube provided.

   c. Using a micropipette, aspirate and discard the preservative buffer from the top of the column. To prevent drying of the column bed, proceed to the next step immediately.

d. Equilibrate the column by adding 250 µl of PBS and allow the liquid to enter the column matrix under gravity. Discard the flow-through buffer.

e. Repeat step 4d once before proceeding to the next step.

C. Purification of exosomes

5. Carefully apply the 100 µl exosome-containing supernatant from step 3 to the top of the column and place the column into the waste tube

6. Allow the liquid to enter the column matrix under gravity. Discard the flow-through.

7. Place the column into a fresh 1.5 ml microcentrifuge tube (not provided). Add 180 µl of PBS to the top of the column.

8. Ensure that the column has fully eluted. Any drops that may be hanging from the column nozzle can be gathered in the sample collection tube by gently tapping the nozzle to the side of the tube.

9. Remove the column from the sample collection tube and discard the column.

10. Briefly centrifuge the sample collection tube containing the isolated exosomes at 100 x *g* for 30 seconds in order to collect all liquid to the bottom of the tube. The isolated exosomes are now ready for downstream applications.

Iterative loading, flushing and storage

For non-concentrated (e.g. precipitated) samples larger than 100 µl iterative loading allows sample volumes up to 500 µl to be loaded. Simply flush the columns with PBS after purification of the first 100 µl of sample to remove free protein from the column and allow additional sample to be purified on the same column.

- Following the elution of your purified exosomes, add a further 4 x 200 µl (800 µl total) PBS. This removes all free protein fractions from previous loadings (as determined by Bradford assay).

- The column may then be loaded with further (or a new) sample by repeating steps 4-10 from the protocol above.

- If storing the column for future use, use 20% ethanol to flush instead of PBS.

Note: Flow rates may be reduced if the column is re-used.

Troubleshooting

My sample does not elute from the column.

- Ensure that the outlet plug has been removed from the base of the column. The outlet plug must be removed before the screw cap.
- 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 number 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. Centrifuging the column at high speed may also compress the resin in the column, making the column inefficient.
- 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.
- Exosome yield is dependent on a variety of factors, particularly the type of biological fluid used as starting material.

My sample has no measurable exosomes.

- This is most likely caused by complete drying out of the column causing loss of functionality. Ensure the columns are kept hydrated at all times.

Can I increase the elution volume?

- It is not recommended as it will result in co-elution of ribonucleoprotein particles.
- As an alternative, the EX05 Exo-spin™ mini-HD kit can be used. The protocol will be performed under gravity and at least two fractions of 200 µl can be collected for further downstream analysis.

I do not have a high-speed centrifuge.

- 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 to spin 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.

I do not have a low-speed centrifuge.

- 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 EX05 Exo-spin™ mini-HD kit can be used. The protocol will be performed under gravity and at least two fractions of 200 µl can be collected for further downstream analysis.

Related products for exosome research

Related products	Product description	Product codes
Exosome detection	**Exosome antigen antibodies**	EX201, EX202, EX204, EX203
	TRIFic™ detection assay	EX101, EX102, EX103
Nanoparticle Tracking Analysis (NTA) size profiling service	**ZetaView® NTA Particle Analysis Service**	ZV-1 and ZV-12
Isolation stands	**Exo-rack - a rack for Exo-spin™ mini and miniHD columns**	EX10-S and EX10-L

TRIFic™ detection assay

The TRIFic™ exosome assay is similar to an ELISA, however, there are some significant differences. Unlike an ELISA, there is no enzymatic reaction. Rather, the target is directly detected with a Europium label. TRIFic™ exosome assays deliver clear, consistent, and quantitative data from purified or unpurified samples, including direct measurement of exosomes from plasma in a convenient 96-well format. TRIFic™ exosome assays are available for the widely-used markers of exosomes, the tetraspanin proteins CD9, CD63 and CD81.

NTA size profiling service

Exosome characterization service for analysis of particle size and numbers using the ZetaView® instrument from Particle Metrix.

Exo-rack

The Exo-rack has been designed specifically for use with Exo-spin™ mini (Cat codes EX01, EX02 and EX03) and Exo-spin™ mini-HD products (Cat code EX06). The Exo-rack is constructed from three separate materials: bioplastic, carbon-reinforced plastic and acrylic. The rack features innovative soft column gripping devices which firmly grip each column in position. The grippers allow the columns to be inserted into the rack from the side enabling rapid set up and easy adjustment of column height as needed.

References

- Witwer KW *et al.* J Extracell Vesicles 2013;2:10.3402/jev.v2i0.20360
- Martins TS *et al.* PLoS One 2018;13(6): e0198820

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