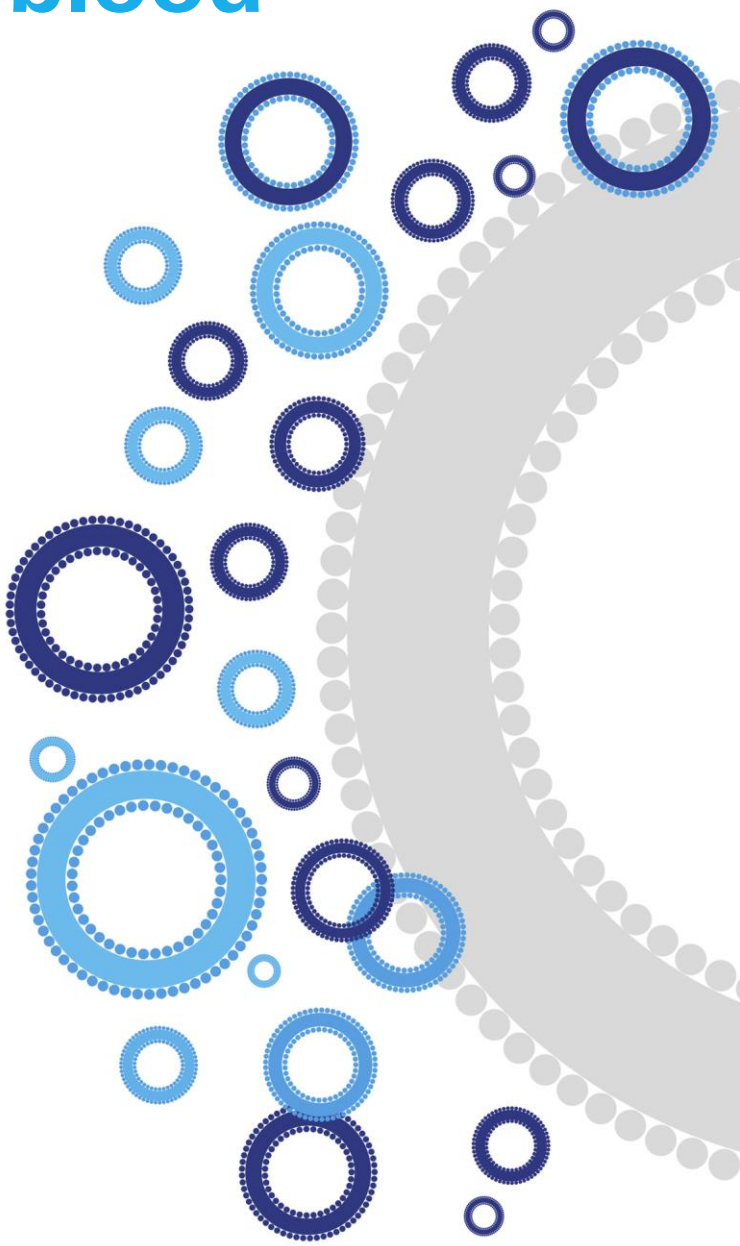
User Guide

# Exo-spin™ mini blood

## Exosome Purification Kit

For blood sera/plasma

Cat EX02



Protocol Version 7.0

# Contents

# Exo-spin™ mini blood Exosome Purification Kit

## Product components

**EX02-8 Exo-spin™ mini blood kit (8 columns)**

- 1 x Exo-spin™ Buffer, 2 ml
- 8 x Exo-spin™ columns with waste collection tubes
- 1 x PBS without calcium chloride and magnesium chloride, 7 ml
- 1 x User Guide

**EX02-25 Exo-spin™ mini blood kit (24 columns)**

- 1 x Exo-spin™ Buffer, 15 ml
- 24 x Exo-spin™ columns with waste collection tubes
- 1 x PBS without calcium chloride and magnesium chloride, 30 ml
- 1 x User Guide

**EX02-50 Exo-spin™ mini blood kit (48 columns)**

- 1 x Exo-spin™ Buffer, 30 ml
- 48 x Exo-spin™ columns with waste collection tubes
- 2 x PBS without calcium chloride and magnesium chloride, 30 ml (total 60 ml)
- 1 x User Guide

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

## Storage

**Upon receipt, store purification columns and Exo-spin™ Buffer at 4°C.**
All other components should be stored at room temperature (15°C – 25°C).

Correctly stored components are stable for at least 6 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.
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 blood samples, from 100 – 500 µl (sera) or 100 – 250 µl (plasma) starting volume per column. Samples less than 100 µl in volume should be diluted with PBS to a final volume of 100 µl, but a low exosome concentration should be expected.

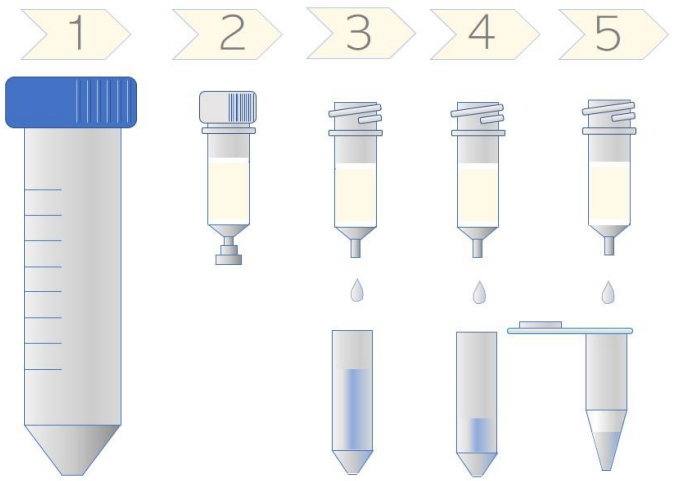
For more information on our exosome isolation range please refer to our website.

# Protocol for purification of intact exosomes from serum or plasma using Exo-spin™ blood

The 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.

The column is designed to elute exosomes from 100 µl liquid. Samples less than 100 µl in volume should be diluted with PBS to a final volume of 100 µl. Sample volumes over 100 µl need to be reduced by precipitation with Exo-spin™ Buffer: per column, a maximum starting sample volume of 500 µl of sera or 250 µl of plasma may be processed with buffer and precipitated exosomes resuspended into 100 µl PBS before loading. For larger sample volumes, use multiple columns per sample or Exo-spin™ midi columns (cat EX04-5/-20).

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 starting blood sample (100 – 250 µl plasma or 100 – 500 µl sera) to a microcentrifuge tube (not supplied with kit) and spin at 300 × *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.
   **For a volume of 100 µl sera or plasma samples, it is possible to omit precipitation steps 3–7 and proceed directly to part C. If precipitation is routinely omitted, Exo-spin™ columns may be purchased separately.**

## B. Precipitate exosome-containing fraction

Note: Precipitating agents can interfere with mass spectrometry in downstream analysis.

3. Transfer supernatant to a new microcentrifuge tube and add Exo-spin™ Buffer in a 1:2 ratio (for example, add 250 µl of Exo-spin™ Buffer to 500 µl supernatant).

4. Mix well by inverting the tube and incubate at 4°C for at least 5 minutes.
   **Alternatively, the sample may be incubated for 1 hour at 4°C. This may generate a small increase in exosome yield.**

5. Centrifuge the mixture at 16,000 x *g* for 30 minutes.
   **Alternatively, the sample may be centrifuged for 1 hour. This may generate small increases in exosome yields.**

6. Carefully aspirate and discard the supernatant.
   **Do not allow the sample to dry as this may cause damage to exosomes.**

7. Resuspend the exosome-containing pellet in 100 µl of PBS (provided).
   **If the pellet does not readily resuspend, warm for 10 minutes at 37°C.**
   **If the pellet is still difficult to resuspend, please refer to the troubleshooting section on page 10.**

## C. Exo-spin™ column preparation

8. 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 first 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 8d once before proceeding to the next step.

## D. Purification of exosomes

9. Carefully apply the 100 µl of resuspended exosome-containing pellet (from step 7) or 100 µl pre-cleared serum (from step 2) to the top of the Exo-spin™ column and place the column into the waste collection tube.

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

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

12. 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.

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

14. 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.

# Protocol for purification of exosomes from CSF using Exo-spin™

This protocol is adapted from Martins *et al*., 2018.

## A. Prepare CSF starting sample

1. Centrifuge CSF at 1000 x *g* for 5 minutes.
2. Transfer supernatant to a new microcentrifuge tube (not supplied with kit) and spin at 16,000 x *g* for 30 minutes at 4ºC. Use 5 ml of CSF sample per column.

## B. Precipitate exosome-containing fraction

3. Transfer supernatant to a new microcentrifuge tube and add Exo-spin™ Buffer in a 2:1 ratio (for example, add 5 ml of Exo-spin™ Buffer to 10 ml supernatant).
4. Mix well by inverting the tube and incubate at 4°C overnight.
5. Centrifuge the mixture at 16,000 x *g* for 1 hour at 4ºC.
6. Carefully aspirate and discard the supernatant.
   **Do not allow the sample to dry as this may cause damage to exosomes.**
7. Resuspend the exosome-containing pellet in 100 µl of PBS (provided).

## C. Exo-spin™ column preparation and purification of exosomes

Please follow steps 8 to 14 on page 6 to purify exosomes from CSF using Exo-spin™ columns.

# 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 8-14 on page 6.
- 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 should be removed before the screw cap.

**My sample contains a lower yield of exosomes than expected.**

- Ensure that the column does not dry out during the procedure.
- Adhere to the volumes indicated for sample addition to the column. If the sample volume or PBS volume to elute is too small, the exosomes will be retained within the column.
- Ensure that precipitation of the exosome-containing pellet is performed for at least 5 minutes at 4°C, and that the Exo-spin™ Buffer has been properly mixed with the sample.
- Exosome yield is dependent on a variety of factors, particularly the type of biological fluid used as starting material and can vary greatly from patient to patient.

**My pellet is particularly difficult to resuspend after the precipitation step. What can I do?**

- Perform the following steps:

  a) Centrifuge the pellet at 1,500 x *g* for 30 minutes, instead of 16,000 x *g* as instructed in step 5.
  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 more easily.

**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.

**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.

# Related products for exosome research

| Related products | Product description | Product code |
|---|---|---|
| **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 |
| **Exosome isolation stand** | **Exo-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 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 EX01, EX02 and EX03) and Exo-spin miniHD products (cat 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 if 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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