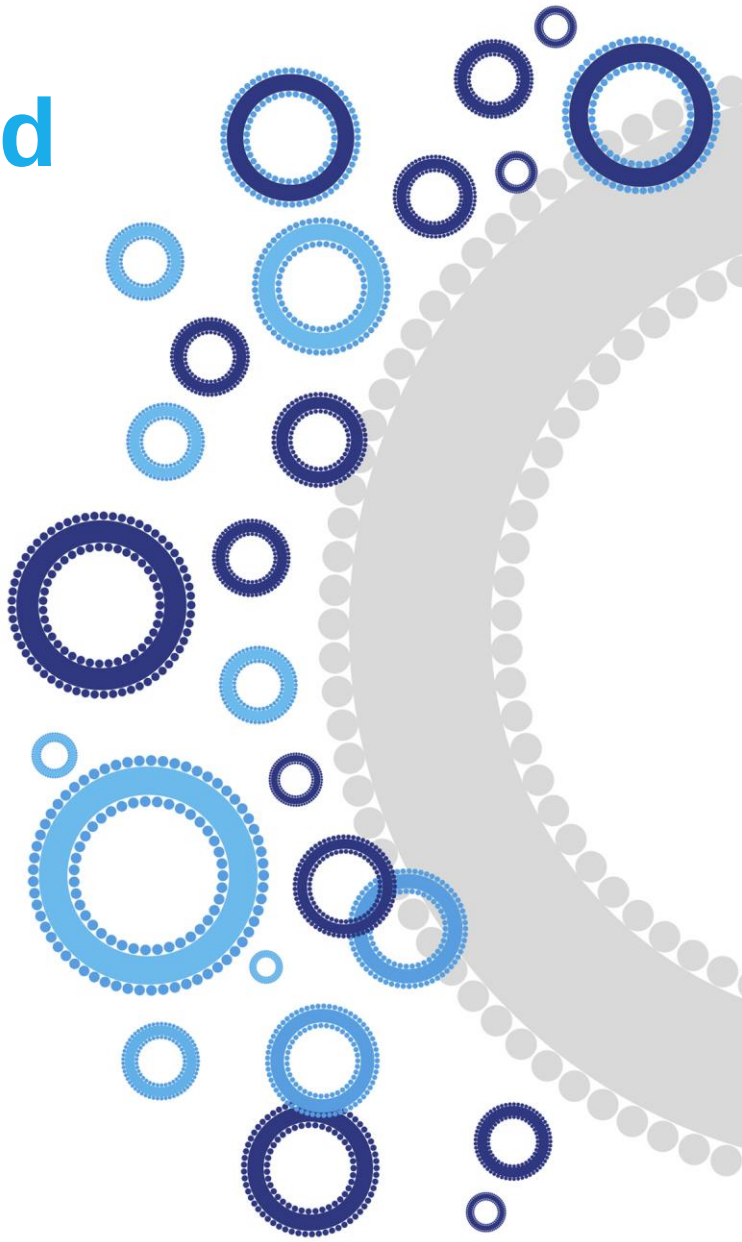
User Guide

Exo-spin™ Blood

Exosome Purification Kit

For blood sera/plasma

Cat EX02



Protocol Version 6.1

Contents

Exo-spin™ Blood Exosome Purification Kit

Product components

EX02-8 Exo-spin™ 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™ 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™ 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
- 1 x User Guide

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

General exosome isolation information

A. Notes on collection of blood samples

Sample collection and handling prior to purification may have a significant impact on the quality of purified exosomes (Witwer *et al*., 2013). It is recommended that exosomes are harvested from plasma, not sera, as 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 should not be used. Exo-spin™ columns can purify samples of 100 µl directly without any need for precipitation. Plasma contains much higher protein levels than sera and can only be processed directly using EX04 Exo-spin™ Midi 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.

The EX02 Exo-spin™ Blood kit may be used for isolation of intact exosomes from blood plasma and sera.

Do not exceed a maximum of 500 µl sera or 250 µl plasma for each column. For 100 µl sera samples, the precipitation step using Exo-spin™ Buffer may be omitted. If you only perform this type of purification, columns are available as a separate product (EX03).

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.

A kit specifically for isolation of exosomes from biological fluids with lower protein and exosome content (EX01 Exo-spin™) is also available.

Protocol for purification of intact exosomes using Exo-spin™ Blood

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 sample volume range of 100 – 500 µl (sera) or 100 – 250 µl (plasma) may be used per column. For larger sample volumes, use multiple columns per sample. For 100 µl sera samples, the precipitation step using Exo-spin™ Buffer may be omitted.

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

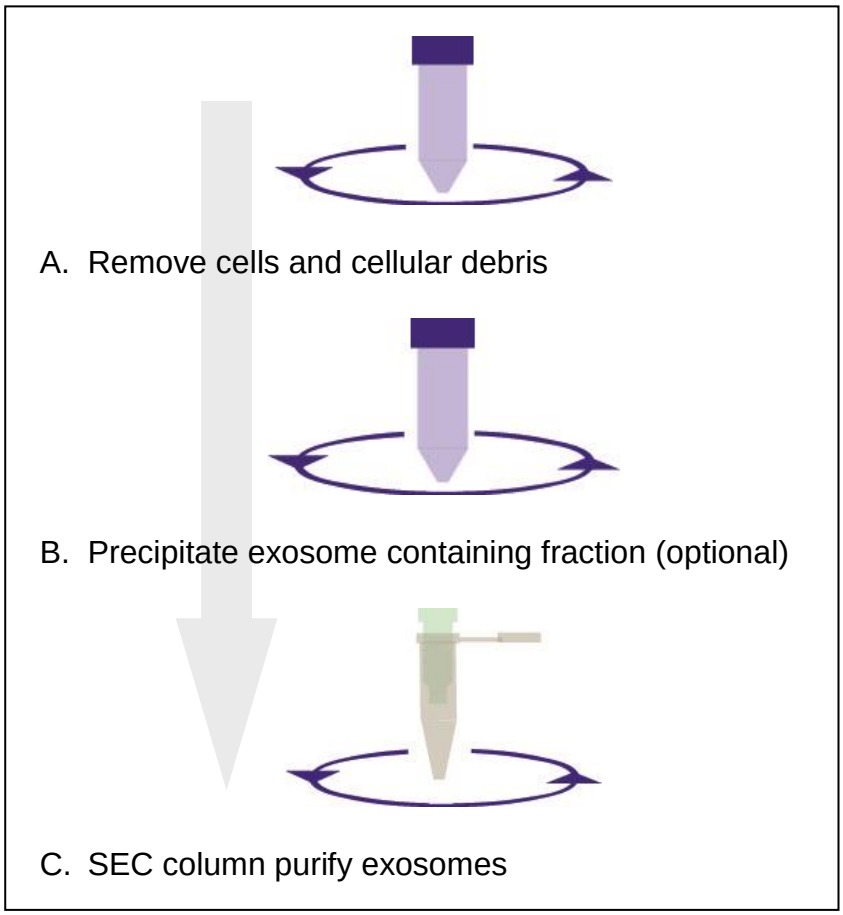


Figure 1. Protocol overview

A. Remove cells and cell debris

1. Transfer starting blood sample (100 – 250 µl plasma or 100 – 500 µl sera) to a microcentrifuge tube and spin at 300 × *g* for 10 minutes to remove cells.

2. Transfer supernatant to a new centrifuge tube (not supplied with kit) and spin at 16,000 × *g* for 30 minutes to remove any remaining cell debris.
   For 100 µl sera samples, omit precipitation steps 3–7 and proceed directly to step Load the supernatant directly on to the column. For downstream mass spectrometry applications, omitting precipitation steps is recommended.

B. Precipitate exosome-containing fraction

3. Transfer supernatant to a new centrifuge 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 5 minutes.
Alternatively, the sample may be incubated for up to an 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 an 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, reduce the amount of starting material.

C. Purification of exosomes

8. Prepare the Exo-spin™ column prior to application of your sample
   a. Remove the outlet plug and place the Exo-spin™ column into the waste collection tube provided.
   The outlet plug must be removed before the screw cap.

   b. 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.

   c. Equilibrate the column by adding 250 µl of PBS and centrifuge at 50 x *g* for 10 seconds.* If any PBS remains above the top filter, repeat spin at the same speed with 5 second increments. Do not spin at excessive speed or for too long as this may desiccate or compress the resin.

   d. Repeat step 8c.
   **An example of a suitable centrifuge is the CappRondo microcentrifuge (Capp®, CR-68X).*

9. Carefully apply the 100 µl of resuspended exosome-containing pellet (from step 7) to the top of the column and place the column into the waste collection tube.

10. Centrifuge at 50 x *g* for 60 seconds. Discard the flow-through.

11. Place the column into a 1.5 ml microcentrifuge tube. Add 200 µl of PBS to the column.

12. Centrifuge at 50 x *g* for 60 seconds to elute the purified exosomes.

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.

Next steps

Next steps	Product description	Product code
Exosome detection	**Exosome antigen antibodies**	EX201, EX202, EX204, EX203
	TRIFic™ detection assay	EX101, EX102, EX103
Exosome tracking	**ExoFLARE™ tracking assay**	EX301, EX302, EX303, EX304, EX305, EX306
NTA size profiling service	**ZetaView NTA Particle Analysis Service**	ZV-1 and ZV-12

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.

ExoFLARE™ tracking assay

ExoFLARE™ utilizes a combination of a FLARE (FLuorescence Activating Response Element) protein tag together with a pro-fluorophore dye. Neither the protein nor dye exhibit fluorescence in isolation. However, when the protein binds to the dye, it causes a change in structure which results in fluorescence. The dye and protein form an unstable bond with a continuous turnover of the dye, resulting in sustained fluorescence without the levels of photo-bleaching associated with fluorescent proteins (i.e. GFP). This enables ExoFLARE™ to be monitored for extensive periods to allow tracking of dye movement.

NTA size profiling service

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

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 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. Centrifuging the column at high speed may also compress the resin in the column, making the column inefficient. If the column dries out, reduce spin speed and/or time.
- 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.
- Ensure that precipitation of the exosome-containing pellet is performed for at least 1 hour 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. If media is used, the amount of exosomes present will vary widely depending on the cell line and the length of exposure (conditioning) of cells to the media.

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?

- This 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.

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).

Reference

- Witwer KW et al. J Extracell Vesicles 2013;2:10.3402/jev.v2i0.20360

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