CELL guidance systems

User Guide

Exo-spin™ Blood

Exosome Purification Kit

For blood sera/plasma

Cat EX02

Protocol Version 5.7

Contents

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.

Product Components

EX02-8 Exo-spin™ blood kit (8 samples) for blood sera/plasma

- 1 x Exo-spin™ Buffer, 2 ml
- 8 x 1.0 ml Exo-spin™ columns with waste collection tubes
- 1 x PBS without calcium chloride and magnesium chloride, 4 ml
- 1 x User Guide

[Not supplied: 1.5 ml microcentrifuge collection tubes.]

EX02-25 Exo-spin™ blood kit (24 samples) for blood sera/plasma

- 1 x Exo-spin™ Buffer, 15 ml
- 24 x 1.0 ml Exo-spin™ columns with waste collection tubes
- 1 x PBS without calcium chloride and magnesium chloride, 30 ml
- 1 x User Guide

[Not supplied: 1.5 ml microcentrifuge collection tubes.]

EX02-50 Exo-spin™ blood kit (48 samples) for blood sera/plasma

- 1 x Exo-spin™ Buffer, 30 ml
- 48 x 1.0 ml Exo-spin™ columns with waste collection tubes
- 1 x PBS without calcium chloride and magnesium chloride, 30 ml
- 1 x User Guide

[Not supplied: 1.5 ml microcentrifuge collection tubes.]

Product Information

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

Do not to exceed a maximum of 0.5 ml of sera or 0.25 ml plasma for each column. For 0.1 ml 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 available.

Proteomic analysis

Precipitants can interfere with mass spectrometry analysis and should not be used. Exo-spin™ columns can purify samples of 0.1 ml 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.

General Information

Note on collection of blood samples

A review in 2013 by Witwer *et al* (Journal of Extracellular Vesicles (2013) 2: 20360) indicates that the way samples are collected and handled prior to purification can have a significant impact on the quality of purified exosomes. This review recommends exosome 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 effect 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).

Protocol for purification of intact exosomes from blood plasma and sera using Exo-spin™ Blood (EX02)

Note

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

A maximum of 0.5 ml sample (sera) or 0.25 ml (plasma) per column may be used. For any larger sample volumes, use multiple columns per sample. For 0.1 ml sera samples, precipitation using Exo-spin™ Buffer may be omitted.

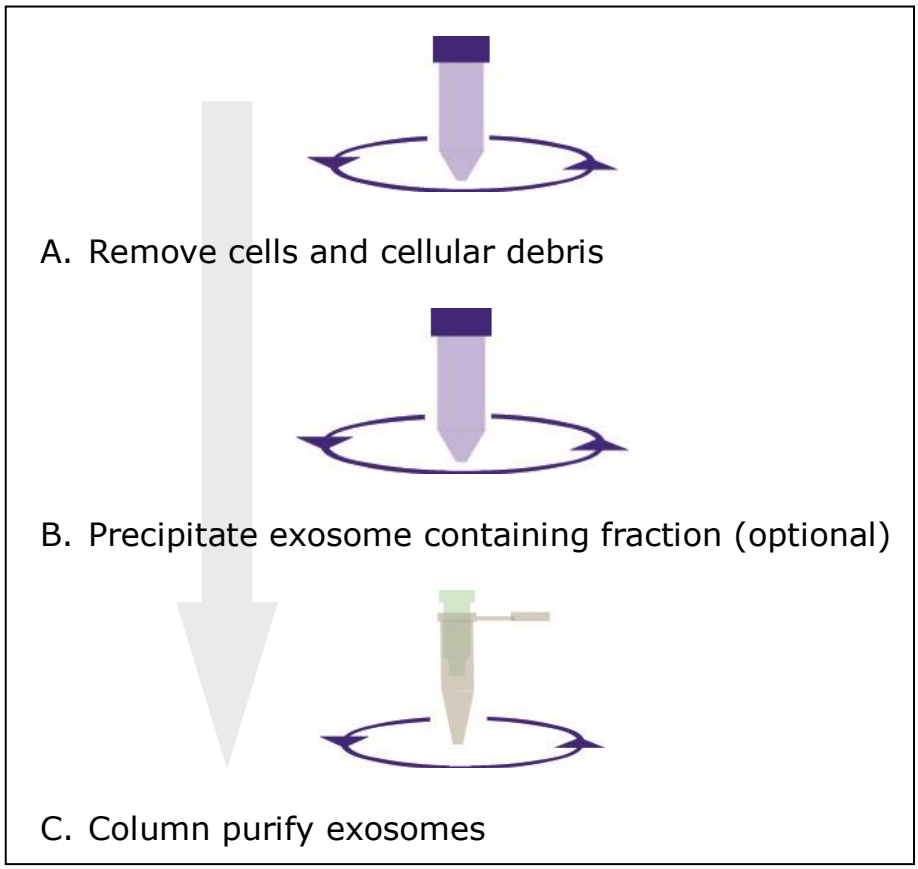

Figure 1 Protocol overview

Protocol

A. Remove cells and cell debris

1. Transfer 0.1 - 0.25 ml of starting sample (blood plasma) or 0.1 - 0.5 ml (blood sera) to a microcentrifuge tube and spin at 300 × g for 10 minutes to remove cells.

For sera samples of 0.1 ml, omit the precipitation step and load the sample directly on to the column (go to Step 8). For downstream mass spectrometry applications, omitting precipitation is recommended.

A web-based tool for calculating centrifugal force (g) and Nomograph are available on the Exo-spin™ product pages of www.cellgs.com.

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

B. Precipitate exosome containing fraction

3 Transfer supernatant to a new microcentrifuge tube and add ½ volume of Exo-spin™ Buffer (for example, add 0.25 ml of Exo-spin™ Buffer to 0.5 ml 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. This may generate small increases in exosome yields.

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.

Note: 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 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. **Outlet plug must be removed before the screw cap.**
- b. Using a micropipette, aspirate preservative buffer from the top of the column and discard it. In order to prevent drying of the column bed, proceed to the next step promptly.
- c. Equilibrate the column by adding 200 µl of PBS (provided) and spin down at 50 x g for 10 seconds.* If any PBS remains above the top filter, repeat spin at the same speed with 5 seconds increments. **Do not spin at too high speed or for too long as this may desiccate or compress the resin.**

* An example of a suitable centrifuge is the iFuge M08 from Neuation Technologies.

9 Carefully apply the exosome containing sample (100 µl from step 7 above) to the top of the column and place the column into the waste tube.

10 Centrifuge at 50 x g for 60 seconds. Discard the eluate.

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

12 Centrifuge at 50 x g for 60 seconds. The 200 µl eluate contains the purified exosomes.

Troubleshooting

My sample does not elute from the column.

Possible causes:

Ensure that the plug has been removed from the base of column.

If the column has been spun too fast, it will be compromised and subsequently not function correctly. Use our online tool and Nomograph, available on the product pages, to calculate the correct RPM for your centrifuge. Be aware that some centrifuges can't provide the required low speeds.

My sample contains a lower amount of exosomes than expected.

Possible causes:

Ensure that the columns do not dry out during the procedure. Any column that is spun for too long or at too high speed may dry out. Spinning the column at too high speed may also compress the resin used in the column. This may cause the column to work inefficiently. If the column dries, reduce spin speed and/or time.

Make sure that the volumes indicated for addition of the sample to the column are adhered to. The exosome containing fraction elutes in a peak as shown below. If the volume of sample added to the column is too small, the exosomes will be retained within the column.

Ensure that at least 1 hour precipitation at 4°C has been allowed and that Exo-spin™ Buffer has been mixed properly with the sample.

The yield of exosomes 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 the media.

My sample has no measurable exosomes.

Possible causes:
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 and proteins.

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