CELL guidance systems

interchim

User Guide

Exo-spin™ Mini-Columns

For rapid purification and clean up of samples up to 100 µl. Suitable for blood sera, cell culture medium and labeled exosome samples

Cat EX03

Protocol Version 2.4

Contents

Storage

Upon receipt, store at 4°C.

Correctly stored components are stable for at least 6 months following purchase.

Product Components

EX03-8

- 8 x 1.0 ml Exo-spin™ columns and waste collection tubes

EX03-25

- 24 x 1.0 ml Exo-spin™ columns and waste collection tubes

EX03-50

- 2 x 24 x 1.0 ml Exo-spin™ columns and waste collection tubes

Product Information

Exo-spin™ columns may be used for isolation of intact exosomes from any sample smaller than 100 µl. This includes blood sera, but not blood plasma. Exo-spin™ columns may be used for purification of samples which have been purified by other methods, such as precipitation and ultracentrifugation, in cases where additional cleanup is required to remove proteins and smaller molecules. Exo-spin™ columns are very effective for the clean-up of labeled exosome samples.

Exosomes isolated using Exo-spin™ columns 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.

Plasma contains much higher protein levels than sera and can only be processed directly using EX04 Exo-spin™ Midi columns. Also, a kit specifically for isolation of exosomes from blood (EX02 Exo-spin™ blood) can be used for isolation of exosomes from plasma.

General Information

Notes on Cell Culture

Fetal bovine serum (FBS) contains a large number of exosomes. Exosome-free FBS should be used in cell culture experiments. Exosome-free FBS is available commercially. Alternatively, we found that Vivaspin20 100,000 MWCO PES (GE Healthcare) centrifugal concentrators or Millipore® UFC910024 Amicon® Ultra-15 Centrifugal Filter Concentrator with Ultracel® 100 Regenerated Cellulose Membrane, NMWL: 100,000 can be used to efficiently remove exosomes from FBS, which should be previously diluted 50% in PBS.

The number of exosomes that are obtained from a cell culture sample will vary depending on a variety of factors. These include the cell line, the length of time the medium is exposed to the cells, and the total number of cells in culture. Cancer cell lines may produce higher numbers of exosomes than non-transformed cell lines.

Note on collection of 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.

Protocol for purification of intact exosomes using Exo-spin™ columns (EX03)

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 100 µl per column may be used.

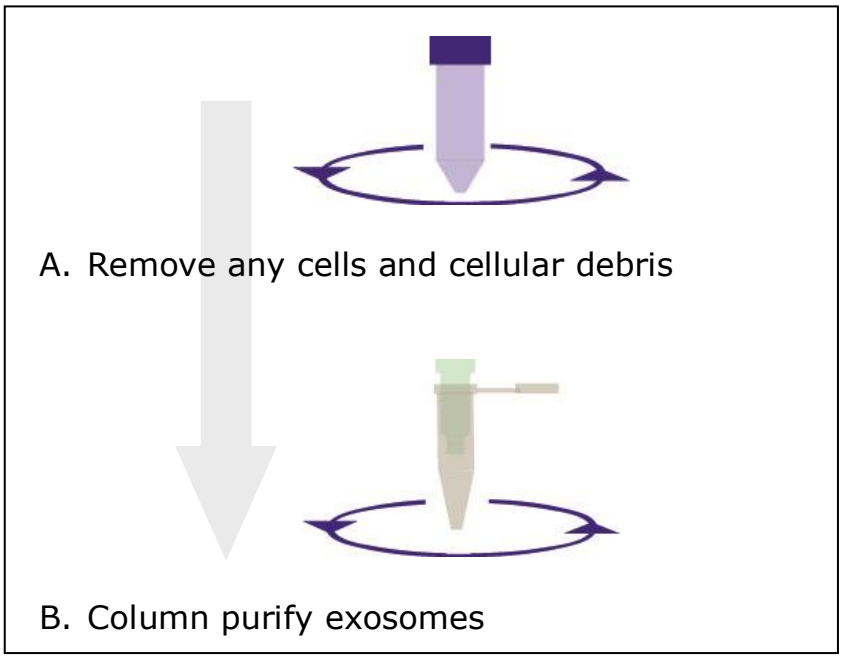

Figure 1 Protocol overview

Protocol

A. For primary samples, such as sera, which contain cells, a step is required to remove cells and cell debris. This step may be omitted for secondary samples being cleaned up which do not contain cells.

1. Transfer 100 µl of starting sample to a centrifuge and spin at 300 × g for 10 minutes to remove cells.

   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 centrifuge tube and spin at 16,000 × g for 30 minutes to remove any remaining cell debris.

B. Purification of Exosomes

3 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 the column bed from drying, proceed to the next step promptly.
   c. Equilibrate the column by adding 200 µl of PBS 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.**

4 Carefully apply the 100 µl exosome-containing sample to the top of the column and place the column into the waste tube. Samples smaller than 100 µl should be made up to 100 µl with PBS prior to applying the sample to the column.

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

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

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

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

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. Adjust the volume of sample to 100 µl with PBS.

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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