CELL guidance systems

User Guide

Exo-spin™ Midi-Columns

Exosome Purification Columns

Cat EX04

Protocol Version 4.9

Contents

1. Storage 2
2. Product Components 2
3. Product Information 3
4. General Information 3
5. Protocol

   Using (EX04) Exo-spin™ midi-columns for purification of whole exosomes 4

6. Troubleshooting 7
7. Purchaser Notification 8

Storage

Upon receipt, store purification columns at 4°C.

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

Product Components

EX04-5

- 5 x 10 ml Exo-spin™ midi-columns with waste collection tubes

EX04-20

- 20 x 10 ml Exo-spin™ midi-columns with waste collection tubes

Product Information

EX04 Exo-spin™ midi-columns may be used for isolation of intact exosomes from cell culture medium, saliva, urine and blood plasma/serum. Other bodily fluids, such as cerebrospinal fluid, breast milk, ascites and nasal secretions have not been tested at the time of writing. However, it is expected that the protocols outlined in this booklet can be adapted for these sample types.

It is recommended not to exceed a maximum of 500 ml of starting material (cell culture medium, saliva) or 1 ml (blood plasma/serum) for each column.

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.

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. 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.

Proteomic analysis

Precipitants can interfere with mass spectrometry analysis and should not be used. Exo-spin™ midi-columns can purify samples of 1 ml directly without any need for precipitation. To maximize the numbers of exosomes that can be purified from media, devices such as CELLine from Integra can increase concentrations of exosomes in media by 7-8 fold.

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 from cell culture medium, urine, saliva and blood plasma/serum using Exo-spin™ midi-columns (EX04)

Note

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

A maximum of 500 ml sample (cell culture medium/urine/saliva) or 1 ml (blood plasma/serum) per column may be used. For any larger sample volumes, use multiple columns per sample. For cell culture media/urine/saliva and blood serum, volumes >1 ml will require precipitation prior to column application. Exo-spin™ Buffer precipitant can be purchased separately (Cat EX06-250).

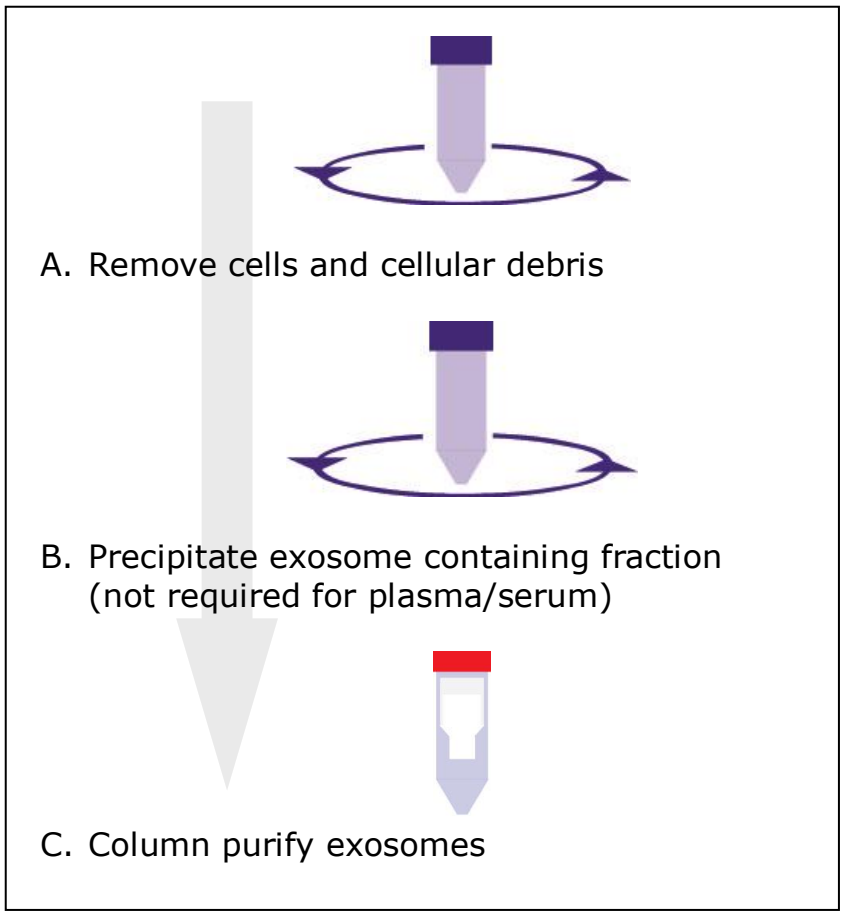

Figure 1 Protocol overview

Protocol

A. Prepare starting sample from cell culture medium, urine, saliva and blood serum

1 Transfer up to 500 ml of starting sample (cell culture medium/urine/saliva) or 1 ml of blood serum to a centrifuge tube(s) and spin at 300 × g for 10 minutes to remove cells.

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

B. Prepare blood plasma starting sample

This protocol is adapted from Welton *et al.* (Journal of Extracellular Vesicles (2015) 4: 27269).

1 Centrifuge blood plasma at 6000 x g for 10 minutes.

2 Filter the resulting platelet-free plasma through a 0.22 µm syringe filter. Separate sample into ≤1 ml aliquots.

C. Precipitate the exosome containing fraction (not required for blood plasma/serum, continue directly to step 8)

3 Transfer supernatant to a new centrifuge tube and add ½ volume of Exo-spin™ Buffer (Cat EX06-250) (for example, add 50 ml of Exo-spin™ Buffer to 100 ml supernatant).

4 Mix well by inverting the tube and incubate at 4°C for at least 1 hr.

Alternatively, the sample may be incubated overnight at 4°C.

5 Centrifuge the mixture at 16,000 x g for 1 hr.

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 1 ml of PBS.

Your sample should now be contained in a volume of 1 ml, regardless of the volume of starting material.

D. Purification of Exosomes

8 Prepare the spin column prior to application of your sample:
   a. Remove the screw cap and discard the preservative buffer. Remove the outlet plug and replace the Exo-spin™ midi-column into the waste collection tube provided. In order to prevent the column bed from drying, proceed to the next step promptly.
   b. Equilibrate the column by sequentially adding 2 x 10 ml of PBS and allow the column to drain under gravity. **Do not centrifuge.**

COLLECTION: USE EITHER QUICK OR HIGH RESOLUTION COLLECTION PROTOCOL

I. Quick Collection Protocol

9 When no buffer remains on the surface of the column, carefully apply the exosome containing sample (1 ml of blood plasma or sample from step 2 or step 7 above) to the top of the column and place the column into the waste tube. Allow the column to drain under gravity.

10 Apply 2 ml PBS to the top of the column. Allow to drain under gravity and discard the waste tube.

11 Place the column into a new 50 ml collection tube. Add a further 3 - 5 ml PBS (Use 3 ml to maximize exosome purity, 5 ml to maximize exosome yield) and collect the eluate.

II. High Resolution Fractionation Protocol

9 When no buffer remains on the surface of the column, place outlet plug back on and fit the column onto a clamp-stand, ready for fraction collection. Carefully apply the exosome containing sample (1 ml of blood plasma or sample from step 2 or step 7 above) in 500 µl increments to the top of the column and start collecting 500 µl fractions.

10 After 2 x 500 µl of sample has been added and two fractions collected, continue by adding 22 x 500 µl PBS and collect each fraction.

11 The majority of the exosomes will elute between fractions 7 and 12. These fractions can be pooled together for obtaining a 3 ml fraction which contains highly pure exosomes. If a higher yield is desirable, 4 further fractions should be collected up to fraction 16. Fraction number 1 is the 500 µL volume eluted after addition of the first 500 µL of sample.

Troubleshooting

My sample contains a lower amount of exosomes than expected.

Possible causes:

Ensure that the columns do not dry out during the procedure.

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

For samples that are precipitated, 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 to the media (conditioning).

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.

Purchaser Notification

Limited warranty Cell Guidance Systems and/or its affiliate(s) warrant their products as set forth in the Terms of Sale found on the Cell Guidance Systems web site at www.cellgs.com/Pages/Terms_and_Conditions.html

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