CELL guidance systems

User Guide

Exo-spin™

Exosome Purification Kit

For cell culture media/urine/saliva

and other low-protein biological fluids

Cat EX01

Protocol Version 5.8

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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

EX01-8 Exo-spin™ kit (8 columns) for cell culture media, urine and saliva For processing a total sample volume of 120 ml

- 2 x Exo-spin™ Buffer, 30 ml
- 8 x 1.0 ml Exo-spin™ columns with waste collection tubes
- 1 x PBS without calcium chloride and magnesium chloride, 4 ml

[Not supplied: large volume (15 ml, 50 ml) centrifuge tubes and 1.5 ml microcentrifuge collection tubes.]

EX01-25 Exo-spin™ kit (24 columns) for cell culture media, urine and saliva For processing a total sample volume of 500 ml

- 1 x Exo-spin™ Buffer, 250 ml
- 24 x 1.0 ml Exo-spin™ columns with waste collection tubes
- 1 x PBS without calcium chloride and magnesium chloride, 30 ml

[Not supplied: large volume (15 ml, 50 ml) centrifuge tubes and 1.5 ml microcentrifuge collection tubes.]

EX01-25L Exo-spin™ kit (24 columns) for cell culture media, urine and saliva For processing a total sample volume of 1000 ml

- 2 x Exo-spin™ Buffer, 250 ml (total 500 ml)
- 24 x 1.0 ml Exo-spin™ columns with waste collection tubes
- 1 x PBS without calcium chloride and magnesium chloride, 30 ml

[Not supplied: large volume (15 ml, 50 ml) centrifuge tubes and 1.5 ml microcentrifuge collection tubes.]

EX01-50 Exo-spin™ kit (48 columns) for cell culture media, urine and saliva For processing a total sample volume of 1000 ml

- 2 x Exo-spin™ Buffer, 250 ml (total 500 ml)
- 48 x 1.0 ml Exo-spin™ columns with waste collection tubes
- 1 x PBS without calcium chloride and magnesium chloride, 30 ml

[Not supplied: large volume (15 ml, 50 ml) centrifuge tubes and 1.5 ml microcentrifuge collection tubes.]

Product Information

EX01 Exo-spin™ may be used for isolation of intact exosomes from cell culture medium, saliva and urine. An alternative protocol is available for processing cerebrospinal fluid using EX01 Exo-spin™, please refer to Welton *et al* (Journal of Extracellular Vesicles (2017) 6(1): 1369805). Other bodily fluids, such as 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 50 ml of starting material for each column. Please note that purchasing additional Exo-spin™ Buffer (EX06-250) is required for processing the aforementioned maximum volume of starting material in all columns.

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.

Serum and plasma contain several orders of magnitude more exosomes compared to other biological fluids. A kit specifically for isolation of exosomes from blood (EX02 Exo-spin™ Blood) is available. However, the components of EX01 are interchangeable and may be used with blood sera and plasma samples.

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) 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™ columns can purify samples of 0.1 ml directly without any need for precipitation. To maximize the numbers of exosomes that can be purified from cell culture 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 and saliva using Exo-spin™ (EX01)

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 50 ml sample (cell culture medium/urine/saliva) per column may be used. For any larger sample volumes, use multiple columns per sample.

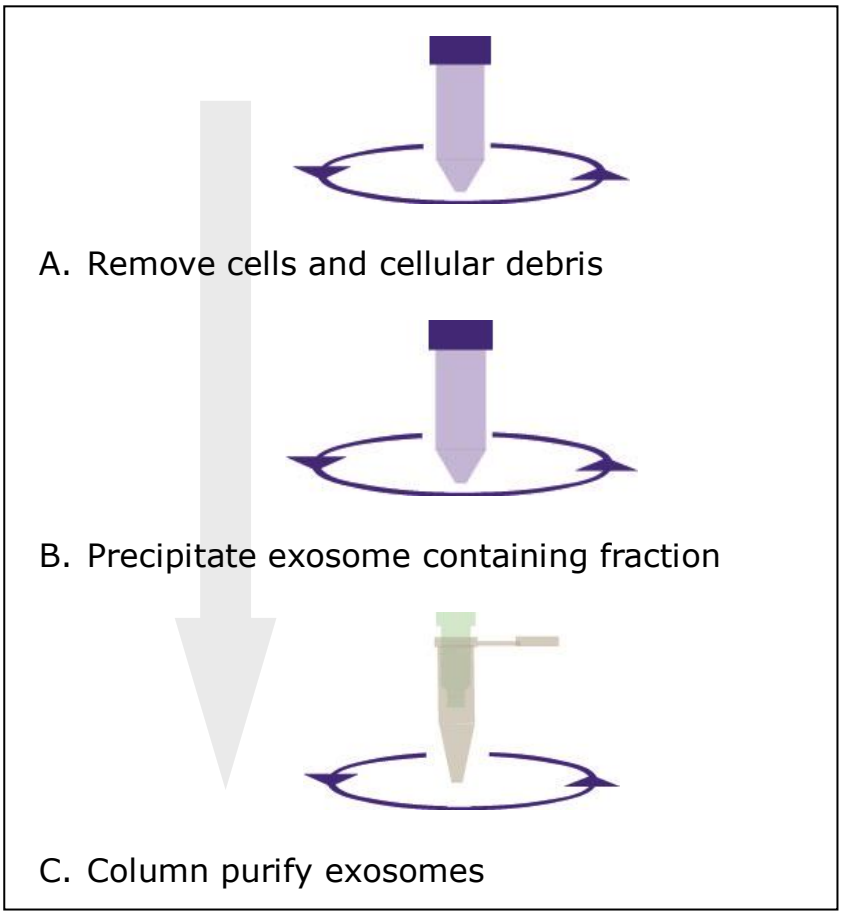

Figure 1 Protocol overview

Protocol

A. Remove cells and cell debris

1. Transfer 1 - 50 ml of starting sample (cell culture medium/urine/saliva) to a centrifuge tube (not supplied with kit) 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 (not supplied with kit) 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 centrifuge tube (not supplied with kit) and add ½ volume of Exo-spin™ Buffer (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 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 100 µl of PBS (provided).

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. **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 pellet (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.

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

If you have any questions, please contact Cell Guidance Systems.

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