CELL guidance systems

interchim

User Guide

Exo-spin™

Exosome Purification Kit

For cell culture media/urine/saliva and other low-protein biological fluids

Cat EX01

Protocol Version 7.0

Contents

Exo-spin™

EX01 Exosome Purification Kits

Note: for larger samples sizes and blood – please see website

Product components

EX01-8 Exo-spin™ kit (8 columns)

- 2 x Exo-spin™ Buffer, 30 ml (total 60 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

EX01-25 Exo-spin™ kit (24 columns)

- 1 x Exo-spin™ Buffer, 250 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

EX01-25L Exo-spin™ kit (24 columns)

- 2 x Exo-spin™ Buffer, 250 ml (total 500 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

EX01-50 Exo-spin™ kit (48 columns)

- 2 x Exo-spin™ Buffer, 250 ml (total 500 ml)
- 48 x Exo-spin™ columns with waste collection tubes
- 2 x PBS without calcium chloride and magnesium chloride, 30 ml (total 60 ml)
- 1 x User Guide

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

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 3 months following purchase.

General exosome isolation information

A. Notes on cell culture

Fetal bovine serum (FBS) contains a large number of exosomes. Exosome-free FBS should be used in cell culture experiments, which can be obtained commercially. Alternatively, Vivaspin® 20 100kDa MWCO Polyethersulfone (GE Healthcare) or Amicon® Ultra-15 Centrifugal Filter Unit (Millipore) can be used to efficiently remove exosomes from FBS diluted 1:1 with PBS.

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

B. Notes on sample collection

Sample collection and handling prior to purification may have a significant impact on the quality of purified exosomes (Witwer et al., 2013).

C. Proteomic analysis

Precipitants can interfere with mass spectrometry analysis and so precipitation should not be used when purifying exosomes if mass spectrometry is to be performed. In such cases, an alternative concentration method should be used instead of precipitation prior to using the Exo-spin™ columns. To maximize the numbers of exosomes that can be purified from cell culture media, devices such as the CELLine Classic bioreactor flask (Sigma) can increase the concentration of exosomes in media by up to 8-fold.

Product information

Exo-spin™ technology combines Precipitation and Size Exclusion Chromatography (SEC), making it superior to techniques that rely solely on precipitation – these result in co-purification of large amounts of non-exosomal proteins and other material as well as carry-over of the precipitant. 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.

This kit has been developed to process different sample types from <1 ml to 50 ml starting volume per column, including cell culture medium, saliva, urine and other low-protein biological fluids. Samples less than 1 ml in volume can be diluted with PBS to a final volume of 1 ml, but a low exosome concentration should be expected.

For more information on our exosome isolation range please refer to our website.

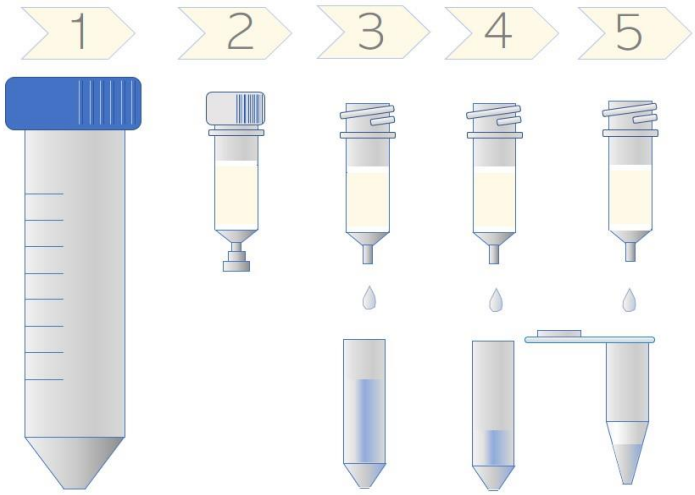
For cerebrospinal fluid (CSF) and human breast milk samples, recommended protocols are available on pages 7, 8 and 9.

Protocol for purification of intact exosomes using Exo-spin™

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 maximum sample volume of 50 ml may be used per column. For larger sample volumes, use multiple columns per sample. Please note that purchasing additional Exo-spin™ Buffer (cat EX06-30 (30 ml) or EX06-250 (250 ml)) is required for processing the aforementioned maximum volume of starting material in all columns.

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



Protocol overview.

A. Remove cells and cell debris

1. Transfer 1 – 50 ml of starting sample to a microcentrifuge tube (not supplied with kit) and spin at 300 × *g* for 10 minutes to remove cells.

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 Exo-spin™ Buffer in a 2:1 ratio (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 hour.
   Alternatively, the sample may be incubated overnight at 4°C. This may generate a small increase in exosome yield.

5. Centrifuge the mixture at 16,000 x *g* for 1 hour.
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).

C. Exo-spin™ column preparation

Note: Exo-rack, a dedicated rack is available from Cell Guidance Systems to hold the mini and min-HD columns and simplify collection tube handling.

Note: This is a significantly updated, simplified protocol which produces excellent results and ensures consistency with samples purified in the Exo-spin 96 well plates.

8. Prepare the Exo-spin™ column prior to application of your sample.

   a. Equilibrate the column temperature by incubating at room temperature for 15 minutes before use.

   b. **Remove the bottom outlet plug before removing the screw cap** and place the Exo-spin™ column into/above the waste collection tube provided.

   c. 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.

   d. Equilibrate the column buffer by adding 250 µl of PBS and allow the liquid to enter the column matrix under gravity. Discard the flow-through buffer.

   e. Repeat step 8d once before proceeding to the next step.

D. Purification of exosomes

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/above the waste collection tube.

10. Allow the liquid to enter the column matrix under gravity. Discard the flow-through.

11. Place the column into a collection 1.5 ml microcentrifuge tube. Add 180 µl of PBS to the top of the column and allow to elute.

12. Ensure that the column has fully eluted. Any drops that may be hanging from the column nozzle can be gathered in the sample collection tube by gently tapping the nozzles to side of tube.

13. Remove the Exo-spin™ column from the sample collection tube.

14. Briefly centrifuge the sample collection tube containing the isolated exosomes at 100 x g for 30 seconds in order to collect all liquid to the bottom of the tube. The isolated exosomes are now ready for downstream applications.

Protocol for purification of exosomes from CSF using Exo-spin™

This protocol is adapted from Martins *et al*., 2018.

A. Prepare CSF starting sample

1. Centrifuge CSF at 1000 x *g* for 5 minutes.

2. Transfer supernatant to a new microcentrifuge tube (not supplied with kit) and spin at 16,000 x *g* for 30 minutes at 4ºC. Use 5 ml of CSF sample per column.

B. Precipitate exosome-containing fraction

3. Transfer supernatant to a new microcentrifuge tube and add Exo-spin™ Buffer in a 2:1 ratio (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 overnight.

5. Centrifuge the mixture at 16,000 x *g* for 1 hour at 4ºC.

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).

C. Exo-spin™ column preparation and purification of exosomes

Please follow steps 8 to 14 on page 6 to purify exosomes from CSF using Exo-spin™ columns.

Protocol for purification of exosomes from human breast milk using Exo-spin™

This protocol is adapted from Rodrigues-Amorim D, Rivera-Baltanás T, Rodriguez-Jamardo C, Fernández-Palleiro P, Olivares JM and Spuch C from the Translational Neuroscience Research Group, Galicia Sur Health Research Institute. University of Vigo, CIBERSAM, Spain.

A. Remove cells and cell debris

1. Transfer 7.5 ml of starting sample to a 15 ml microcentrifuge tube (not supplied with kit) and spin at 300 × *g* for 10 minutes to remove cells.

2. Transfer the supernatant to a new microcentrifuge tube and spin at 16,000 × *g* for 30 minutes to remove any remaining cell debris.
   Transfer the entire supernatant including the layer of milk fat.

B. Precipitate exosome-containing fraction

3. Transfer supernatant to a new centrifuge tube and add Exo-spin™ Buffer in a 1:1 ratio (for example, add 7.5 ml of Exo-spin™ Buffer to 7.5 ml supernatant).

4. Mix well by inverting the tube and incubate at 4°C overnight using a tube rotator.

5. Centrifuge the mixture at 16,000 x *g* for 1 hour at 4ºC.

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 200 µl of PBS (provided).

C. Exo-spin™ column preparation and purification of exosomes

Please follow steps 8 to 14 on page 6 to purify exosomes from CSF using Exo-spin™ columns.

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.

My sample contains a lower amount of exosomes than expected.

- Ensure that the column does not dry out during the procedure.
- 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 higher levels of non-exosomal proteins which will elute from the column after exosomes.

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.

Related products

Related products	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
Nanoparticle Tracking Analysis (NTA) size profiling service	**ZetaView NTA Particle Analysis Service**	ZV-1 and ZV-12
Exosome isolation auxiliary equipment	**Exo-rack set of racks for Exo-spin mini and miniHD columns**	EX10-S and EX10-L
High-throughput exosome isolation	**Exo-spin™ exosome isolation in a 96 well format**	EX07-96

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 the widely-used markers of exosomes, the tetraspanin proteins CD9, CD63 and CD81.

NTA size profiling service

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

Exo-rack

The Exo-rack has been designed specifically for use with Exo-spin mini (Cat codes EX01, EX02 and EX03) and Exo-spin miniHD products (Cat code EX06). The Exo-rack is constructed from three separate materials: bioplastic, carbon-reinforced plastic and acrylic. The rack features innovative soft column gripping devices which firmly grip each column in position. The grippers allow the columns to be inserted into the rack from the side enabling rapid set up and easy adjustment of column height if needed.

Exo-spin™ 96

Exo-spin™ 96 columns have exactly the same internal dimensions as our popular single columns (Cat EX01, EX02, 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.

References

- Witwer KW *et al.* J Extracell Vesicles 2013;2:10.3402/jev.v2i0.20360
- Martins TS *et al.* PLoS One 2018;13(6): e0198820

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Cell Guidance Systems' reagents and services enable control, manipulation and monitoring of the cell, both *in vitro* and *in vivo*

Growth Factors
- Recombinant
- Sustained Release

Exosomes
- Purification
- Detection
- Tracking
- NTA Service

Small Molecules

Cell Counting Reagent

Matrix Proteins

Cell Culture Media
- Photostable
- Custom Manufacturing Service

Cytogenetics Analysis

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