

User Guide

Exo-spin™

Exosome Purification Kit

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

Cat EX01

Protocol Version 6.7



Contents

Product components	3
General exosome isolation information	4
A. Notes on cell culture	4
B. Notes on sample collection	4
C. Proteomic analysis.....	4
Product information	4
Protocol for purification of intact exosomes using Exo-spin™	6
A. Remove cells and cell debris	6
B. Precipitate exosome-containing fraction	6
C. Exo-spin™ column preparation.....	7
D. Purification of exosomes.....	7
Protocol for purification of exosomes from CSF using Exo-spin™	7
A. Prepare CSF starting sample.....	7
B. Precipitate exosome-containing fraction	7
C. Exo-spin™ column preparation and purification of exosomes.....	8
Protocol for purification of exosomes from human breast milk using Exo-spin™	8
A. Remove cells and cell debris	8
B. Precipitate exosome-containing fraction	8
C. Exo-spin™ column preparation.....	8
D. Purification of exosomes.....	9
Storage	9
Related products	9
TRIFic™ detection assay	9
ExoFLARE™ tracking assay	10
NTA size profiling service.....	10
Troubleshooting	10
References.....	11
Purchaser Notification	11

Exo-spin™

Exosome Purification Kit

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.

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 CELLline 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.

This protocol is centrifugation-based. As an alternative, Exo-spin™ mini-HD columns (cat EX05) can be used if a gravity-based protocol is preferred. For more information on our exosome isolation range, a selection guide is available page 5.

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

Table. Exo-spin™ selection guide.

Cell culture medium Saliva Urine Human breast milk* Cerebrospinal fluid* Any low protein biological fluids (1x10 ⁹ particles/ml)		Blood samples (plasma and sera) [†]			
Sample starting volume (per Exo-spin™ column)	Isolation method	Exo-spin™ kit	Sample starting volume (per Exo-spin™ column)	Isolation method	Exo-spin™ kit
<1 ml to 50 ml	Precipitation + SEC (Centrifugation)	EX01	Sera		
<1 ml to 75 ml	Precipitation + SEC (Fractionation by gravity flow)	EX05	≤100 µl	SEC (Centrifugation)	EX03
75 ml to 500 ml	Precipitation + SEC (Fractionation by gravity flow)	EX04	≤150 µl	SEC (Fractionation by gravity flow)	EX05
			<100 µl to 500 µl	Precipitation + SEC (Centrifugation)	EX02
			1 ml	SEC (Fractionation by gravity flow)	EX04
			Plasma		
			≤150 µl	SEC (Fractionation by gravity flow)	EX05
			<100 µl to 250 µl	Precipitation + SEC (Centrifugation)	EX02
			1 ml	SEC (Fractionation by gravity flow)	EX04

* For cerebrospinal fluid and human breast milk samples, validated protocols are available for EX01 only.

† Highly concentrated exosome samples (e.g. 1x10¹² particles/ml) other than blood can also be used.

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.

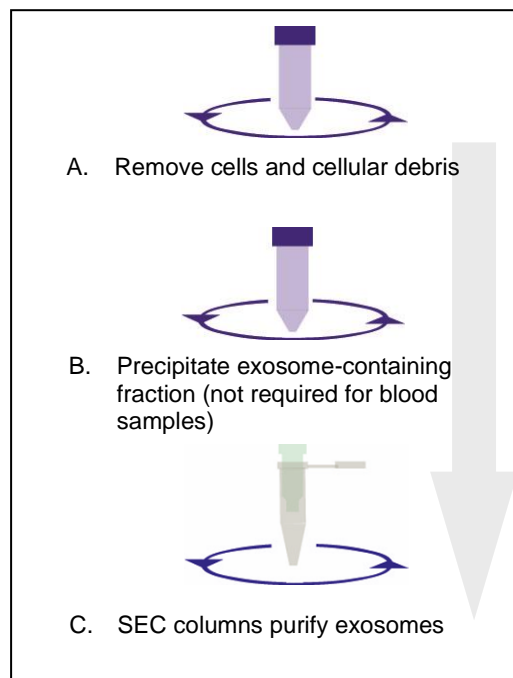


Figure. Protocol overview.

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 × *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 μ l of PBS (provided).

C. Exo-spin™ column preparation

8. Prepare the Exo-spin™ column prior to application of your sample.
 - a. Equilibrate the column at room temperature for 15 minutes before use.
 - b. **Remove the outlet plug before the screw cap** and place the Exo-spin™ column into 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 by adding 250 μ l of PBS and centrifuge at 50 x *g* for 10 seconds.* If any PBS remains above the top filter, repeat spin at the same speed for 5 seconds and repeat as many times as is necessary. Do not spin at excessive speed or for too long as this may desiccate or compress the resin. *An example of a suitable centrifuge is the CappRondo microcentrifuge (Capp®, CR-68X)
 - e. Repeat step 8d once before proceeding to the next step.

D. Purification of exosomes

9. Carefully apply the 100 μ l of resuspended exosome-containing pellet (from step 7) to the top of the column and place the column into the waste collection tube.
10. Centrifuge at 50 x *g* for 60 seconds. Discard the flow-through.
11. Place the column into a 1.5 ml microcentrifuge tube. Apply 200 μ l of PBS to the top of the column.
12. Centrifuge at 50 x *g* for 60 seconds to elute the purified exosomes.

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* at 4°C. 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 4°C.
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 12 on page 7 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 x *g* for 10 minutes to remove cells.
2. Transfer the supernatant to a new microcentrifuge tube and spin at 16,000 x *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 4°C.
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

8. Prepare the Exo-spin™ column prior to application of your sample.
 - a. Equilibrate the column at room temperature for 15 minutes before use.
 - b. **Remove the outlet plug before the screw cap** and place the Exo-spin™ column into 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 by adding 250 µl of PBS and centrifuge at 50 x *g* for 10 seconds.* If any PBS remains above the top filter, repeat spin at the same speed for 5 seconds and repeat as many times as is necessary. Do not spin at excessive speed or

for too long as this may desiccate or compress the resin. *An example of a suitable centrifuge is the CappRondo microcentrifuge (Capp®, CR-68X).

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

D. Purification of exosomes

9. Carefully apply the 200 µl of resuspended exosome-containing pellet (from step 7) to the top of the column and place the column into the waste collection tube.
10. Centrifuge at 50 x g for 5 minutes and discard the flow-through. If supernatant remains above the top filter, centrifuge at 50 x g for an additional 5 minutes to ensure the supernatant enters the column.
11. Place the column into a 1.5 ml microcentrifuge tube. Add 200 µl of PBS to the column.
12. Centrifuge at 50 x g for 60 seconds to elute the purified exosomes.

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.

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

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.

ExoFLARE™ tracking assay

ExoFLARE™ utilizes a combination of a FLARE (FLuorescence Activating Response Element) protein tag together with a pro-fluorophore dye. Neither the protein nor dye exhibit fluorescence in isolation. However, when the protein binds to the dye, it causes a change in structure which results in fluorescence. The dye and protein form an unstable bond with a continuous turnover of the dye, resulting in sustained fluorescence without the levels of photo-bleaching associated with fluorescent proteins (i.e. GFP). This enables ExoFLARE™ to be monitored for extensive periods to allow tracking of dye movement.

NTA size profiling service

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

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.
- If the column has been centrifuged at excessive speed, it will be compromised and subsequently not function correctly. Be aware that some centrifuges cannot provide the low speed required.

My sample contains a lower amount of exosomes than expected.

- Ensure that the column does not dry out during the procedure. Any column that is spun for too long or at excessively high speed may dry out. Centrifuging the column at high speed may also compress the resin in the column, making the column inefficient.
- 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?

- It is not recommended as it will result in co-elution of ribonucleoprotein particles.
- As an alternative, the EX05 Exo-spin™ mini-HD kit can be used. The protocol will be performed under gravity and at least two fractions of 200 µl can be collected for further downstream analysis.

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 $1.68*30 \text{ mins} = 50.4 \text{ minutes}$.

I do not have a low-speed centrifuge.

- It is important to spin at 50 x g as the resin can easily get compressed at even 100 x g. An example of a low-speed centrifuge is CappRondo microcentrifuge (Capp®, CR-68X).
- As an alternative, the EX05 Exo-spin™ mini-HD kit can be used for the same biofluid and starting material than EX01 kit, the protocol will be performed under gravity and therefore no specific material is required.

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

- Pluripotent Stem Cells
- Photostable
- *In Vitro* Blastocyst Culture
- ETS-embryo Culture
- Custom Manufacturing Service

Gene Knock-Up System

Cytogenetics Analysis



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