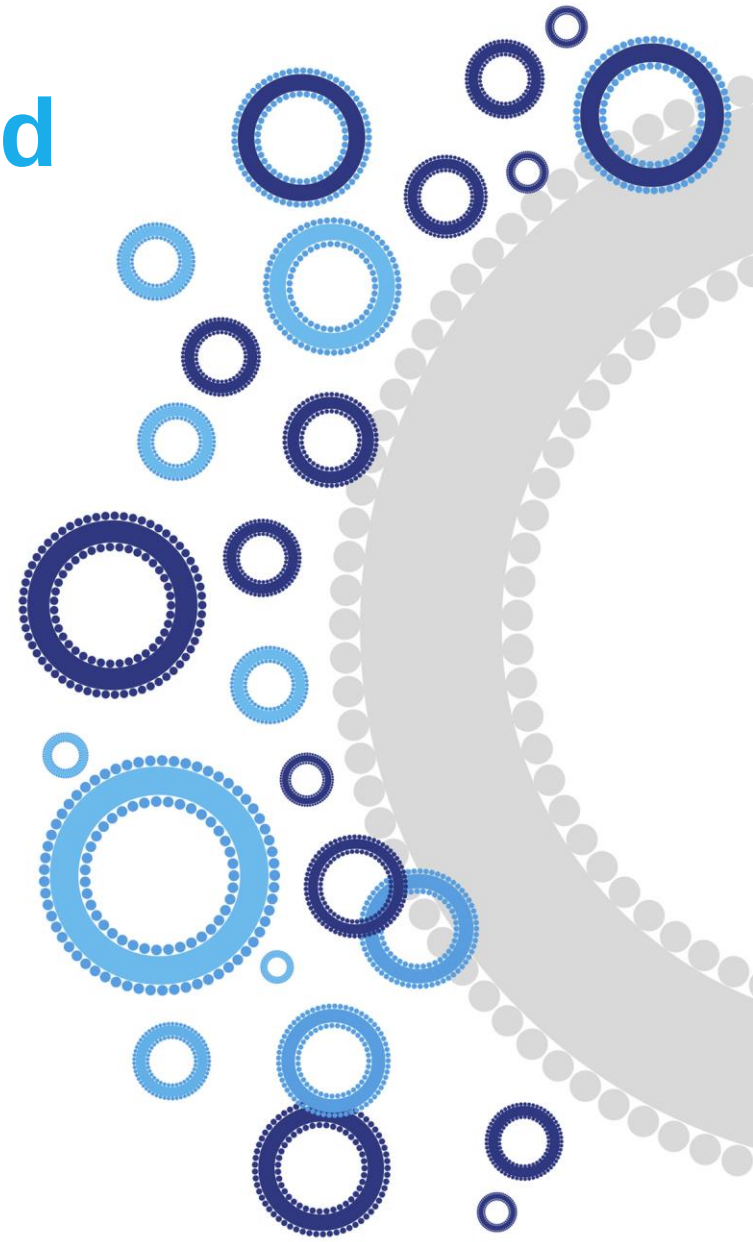
User Guide

Exo-spin™ blood

Exosome Purification Kit

For blood sera/plasma

Cat EX02



Protocol Version 6.8

Contents

Exo-spin™ blood Exosome Purification Kit

Product components

EX02-8 Exo-spin™ blood kit (8 columns)

- 1 x Exo-spin™ Buffer, 2 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

EX02-25 Exo-spin™ blood kit (24 columns)

- 1 x Exo-spin™ Buffer, 15 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

EX02-50 Exo-spin™ blood kit (48 columns)

- 1 x Exo-spin™ Buffer, 30 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 blood samples collection

Sample collection and handling prior to purification may have a significant impact on the quality of purified exosomes (Witwer *et al*., 2013). Sera can contain many platelet-derived exosomes released after clot formation. Use of heparin-based anticoagulants is discouraged because of possible effects on downstream applications (e.g. PCR).

Platelet-derived exosomes may be released from platelets by the physical forces associated with the blood sampling procedure. Standardization of sampling site, needle gauge (wider may be better), and other variables is recommended. To ensure the sample is not contaminated by skin fibroblasts, it has been suggested that the first few milliliters of drawn blood should be discarded.

Collected blood should be handled gently and processed rapidly (within 30 minutes of drawing).

B. 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.

Product information

Exo-spin™ technology combines Precipitation and Size Exclusion Chromatography (SEC), making it superior to techniques that rely solely on precipitation which 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 blood samples, from 100 – 500 µl (sera) or 100 – 250 µl (plasma) starting volume per column. Samples less than 100 µl in volume should be diluted with PBS to a final volume of 100 µl, 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 6.

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. 1x10^{12} particles/ml) other than blood can also be used.

Protocol for purification of intact exosomes using Exo-spin™ blood

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 500 µl sera or 250 µl plasma 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.

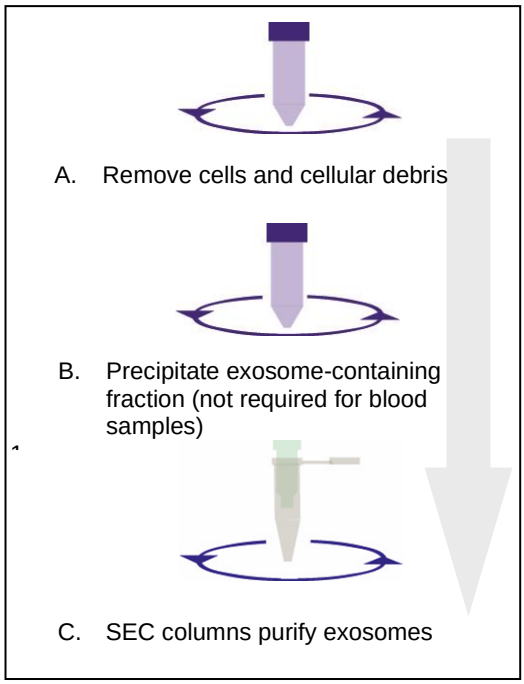


Figure. Protocol overview.

A. Remove cells and cell debris

1. Transfer starting blood sample (100 – 250 µl plasma or 100 – 500 µl sera) 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.
   For 100 µl sera samples, omit precipitation steps 3–7 and proceed directly to part C.

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 250 µl of Exo-spin™ Buffer to 500 µl supernatant).

4. Mix well by inverting the tube and incubate at 4°C at least 5 minutes.
   Alternatively, the sample may be incubated for 1 hour at 4°C. This may generate a small increase in exosome yield.

5. Centrifuge the mixture at 16,000 x *g* for 30 minutes.
   Alternatively, the sample may be centrifuged for 1 hour. This may generate small increases in exosome yields.

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).
   If the pellet does not readily resuspend, warm for 10 minutes at 37°C.
   If the pellet Is still difficult to resuspend, please refer to the troubleshooting section on page 10.

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 100 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 60 seconds. Discard the flow-through.

11. Place the column into a 1.5 ml microcentrifuge tube. Add 200 µl of PBS to the top of 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 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 5 minutes 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.

My pellet is particularly difficult to resuspend after the precipitation step. What can I do?

- Perform the next steps:

a) Centrifuge the pellet at 1,500 x *g* for 30 minutes, instead of 16,000 x *g* as instructed in step 5.
b) Aspirate supernatant.
c) Incubate the pellet for 10 minutes at 37°C.
d) Expel warmed PBS onto the pellet to break it up.
e) Resuspend the pellet using a pipette tip that has been cut to about 1/3rd of the way up to stop pellet blocking the pipette tip.

- If still necessary, lower your starting volume to resuspend the pellet easier.

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 mins = 50.4 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 Exo-spin™ kit, the protocol will be performed under gravity and therefore no specific material is required.

Reference

- Witwer KW *et al.* J Extracell Vesicles 2013;2:10.3402/jev.v2i0.20360

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.

This product incorporates licensed technologies. The purchase of this product conveys to the purchaser the limited, nontransferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed. This product is for internal research purposes only and is not for use in commercial services of any kind, including, without limitation, reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact info@cellgs.com.

CELL GUIDANCE SYSTEMS AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL CELL GUIDANCE SYSTEMS AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

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

General info@cellgs.com
Technical Enquiries tech@cellgs.com
Quotes quotes@cellgs.com
Orders order@cellgs.com

www.cellgs.com

EUROPE
Cell Guidance Systems Ltd
Maia Building
Babraham Bioscience Campus
Cambridge
CB22 3AT
United Kingdom
T +44 (0) 1223 967316
F +44 (0) 1223 750186

USA
Cell Guidance Systems LLC
Helix Center
1100 Corporate Square Drive
St. Louis
MO 63132
USA
T 760 450 4304
F 314 485 5424