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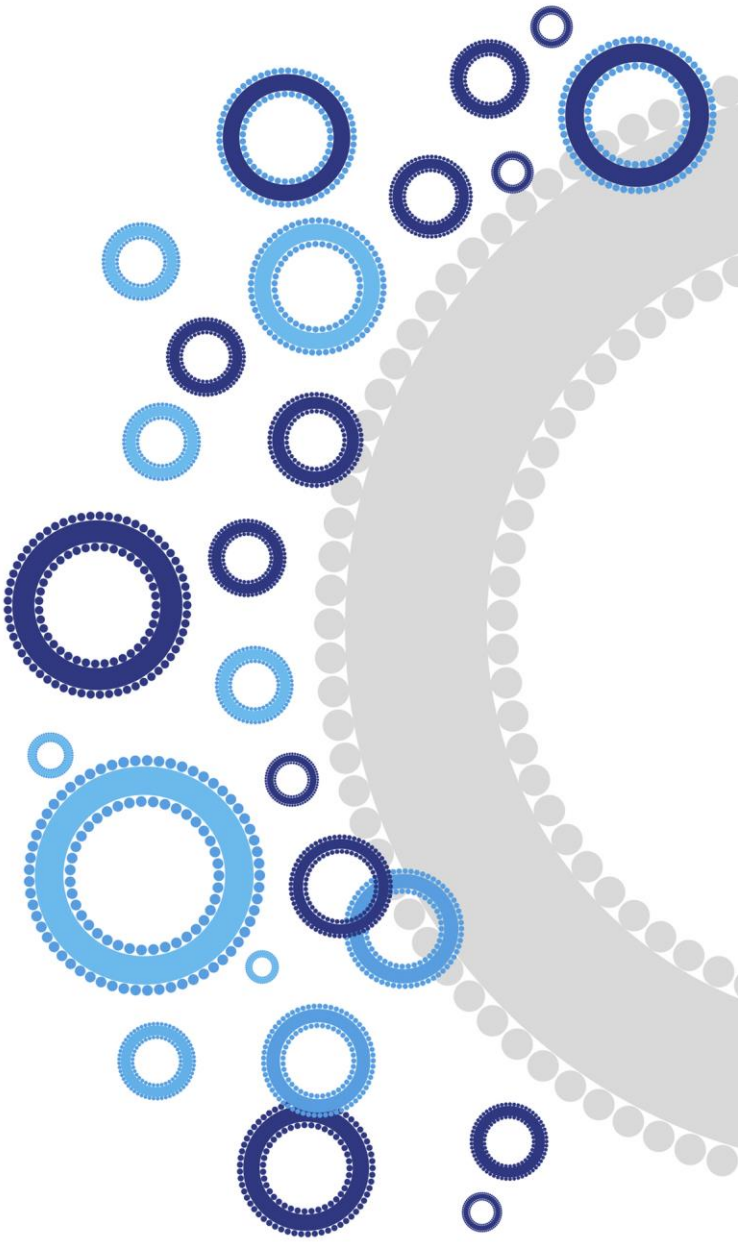
User Guide

Exo-spin™ 96

Exosome Purification Kit

For blood sera/plasma

Cat EX07



Protocol Version 1.1

Contents

Exo-spin™ 96 Exosome Purification Kit

Product components

EX07-96 Exo-spin™ 96 (96 well plate)

- 1 x Exo-spin™ 96 plate
- 1 x 96 deep well waste collection plate
- 1 x 96 well hydrophobic sample collection plate and sealing film
- 1 x Quick Guide

Storage

Upon receipt, store at 4°C.

Correctly stored components are stable for at least 3 months following purchase.

General information for collecting blood samples

Sample collection and handling techniques used prior to purification can have a significant impact on the quality of purified exosomes (Witwer *et al*., 2013). 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 millilitres of drawn blood should be discarded.

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).

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

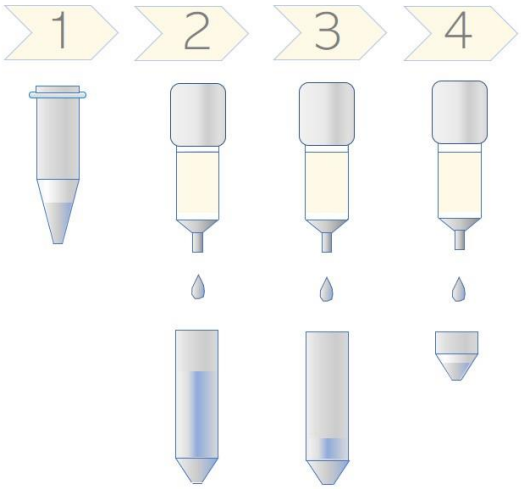
Product information

The columns in the Exo-spin™ 96 plate have exactly the same internal dimensions as Exo-spin mini 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.

This kit has been developed to process blood samples, 100-500 µl sera or plasma volume per column. Samples smaller than 100 µl in volume should be diluted with PBS to a final volume of 100 µl.

For more information on other products in our exosome isolation range please refer to our website http://www.cellgs.com

Protocol for purifying exosomes using Exo-spin™ 96



Protocol overview (1) centrifuge sample to pellet cells (2) Equilibrate column (3) Add sample (4) Elute purified exosomes

The Exo-spin™ columns are equilibrated with ultra-pure water containing 20% ethanol for column stability and sterility. The column matrix should be equilibrated with PBS prior to use.

A maximum sample volume of 500 µl sera or plasma may be used per column. For samples larger than 100 µl, use iterative loading (see page 6). Alternatively, use multiple columns per sample, or concentrate the sample prior to loading (e.g. using a spin-concentrator or a precipitation buffer).

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

A. Remove cells and cell debris

1. Transfer starting blood sample (100 µl sera or plasma) to a microcentrifuge tube (not supplied with the kit) and spin at 300 × *g* for 10 minutes for sera or at 5000 x g for 10 minutes for plasma to remove cells.

2. For sera, transfer supernatant to a new microcentrifuge tube and spin at 16,000 × *g* for 30 minutes to remove any remaining cell debris. For plasma, proceed with the supernatant to the next section.

B. Exo-spin™ 96 plate preparation

3. Prepare the Exo-spin™ 96 plate prior to application of your sample.

   NOTE: Individual strips of 8 columns may be separated from the plate. See section “Column Plate disassembly” below.

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

   b. Remove the lower outlet silicone sealing mat and place the Exo-spin™ 96 plate into the deep-well waste collection plate provided.

   c. Remove the top sealing mat.

   d. Allow the storage buffer to enter and pass through the columns.

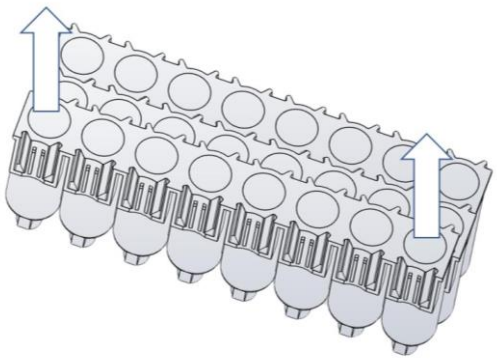
   e. Equilibrate columns by adding 250 µl of PBS and allow to enter the columns.

   f. Repeat step 3e once before proceeding to the next step.

C. Purification of exosomes

4. Apply 100 µl of sample to the top of each column and allow the sample to enter the column.
5. **Optional step to concentrate purified exosomes: Apply 80 µl PBS. Allow to enter column.**
6. Remove any drops hanging from column nozzles by briefly resting the plate on a lint-free tissue.
7. Discard the waste collection plate.
8. Place the columns onto the provided sample collection plate. Add 180 µl* of PBS to the top of each column and allow to elute**.** Larger or smaller volumes of PBS can be added which will affect yield and purity. See section “Elution volume” below. ***If step 5 has been included, reduce this volume to 100 µl.**
9. Ensure that all columns have fully eluted. Any drops that may be hanging from the column nozzles can be gathered in the sample collection plate by gently tapping the nozzles to side of wells.
10. Remove the Exo-spin™ 96 plate from the sample collection plate.
11. Briefly centrifuge the sample collection plate containing the isolated exosomes at 100 x g for 30 seconds in order to collect any liquid to the bottom of the wells. The isolated exosomes are now ready for downstream applications.
12. To seal the plate, use the self-adhesive sealing film provided with the kit. The columns may be recycled to allow larger sample columns to be purified by iterative loading. See “iterative loading”

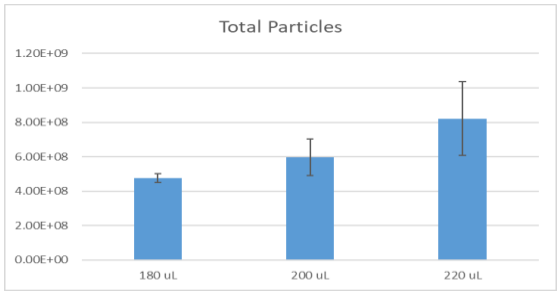
Exo-spin™ 96 plate disassembly

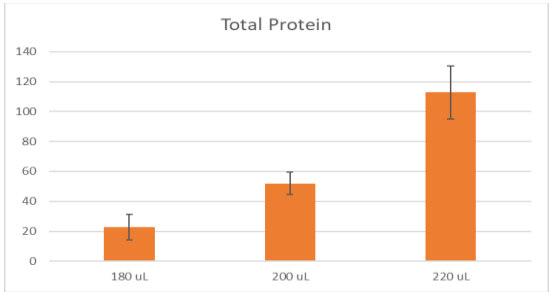


1. The strips of columns interlock via protruding and recessed (male and female) joints. Identify the end of the plate with recessed joints visible and remove the top sealing mat to reveal the number of column strips that are required.
2. Push up the columns to separate as shown.
3. Ensure that unused columns are sealed and contain storage buffer on top.

Elution volume

The elution volume can be modulated depending on downstream requirements.





100 µl FBS purified using Exo-spin™ 96 with elution volumes of either 180 µl, 200 µl or 220 µl showing (left) the total exosome particle numbers (NTA analysis) and (right) total protein (Bradford assay). n=8

Iterative loading, flushing and storage

For non-concentrated (e.g. precipitated) samples larger than 100 µl iterative loading allows sample volumes up to 500 µl to be loaded. Simply flush the columns with PBS after purification of the first 100 µl of sample to remove free protein from the column and allow additional sample to be purified on the same column

- After step11 (Section C) above, add a further 4 x 200 µl (800 µl total) PBS. This removes all free protein fractions from previous loadings (as determined by Bradford assay).
- The column may then be loaded with further (or a new) sample by repeating steps 4-11 above.
- If storing the column for future use, use 20% ethanol to flush instead of PBS

Troubleshooting

My sample contains a lower number of exosomes than expected.

- Ensure that the columns do 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.

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 possible but will result in co-elution of higher levels of non-exosomal proteins (see above).

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 spinning 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
	ExoLISA™ detection assay	EX501, EX502, EX503
Nanoparticle Tracking Analysis (NTA) size profiling service	ZetaView NTA Particle Analysis Service	ZV-1 and ZV-12

ExoLISA™ detection assay

The ExoLISA™ 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 labelled antibody. ExoLISA™ 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. ExoLISA™ exosome assays are available for widely-used markers of exosomes, the tetraspanin proteins CD9, CD63 and CD81.

NTA size profiling service

Cell Guidance Systems provides a cost-effective exosome characterization service for analysis of particle size and particle concentration using the ZetaView instrument from Particle Metrix. For further details, please see our website.

Exo-rack

The Exo-rack has been designed specifically for use with Exo-spin™ mini (Cat codes EX01, EX02 and EX03) and Exo-spin™ mini-HD 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 as needed.

Purchaser Notification

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