User Instructions

ExoEZ™

Exosome Purification Column Kit

Cat#: Exo-A300 (mini)
Cat#: Exo-B300 (midi)
Cat#: Exo-C300 (maxi)

Store kit at +4°C to +8°C on receipt

Product Description

This product (**Cat#: Exo-A300, Exo-B300, or Exo-C300**) is suitable for separating exosome from salt, lipids, proteins and other small nano-particles in sera or plasma or other biofluids. It is also good for the further purification of exosomes isolated from cell culture media and other biofluids using our ExoEZ isolation kit.

Two possible application protocols: gravity and spin, are provided. There is a slightly higher recovery capacity using gravity protocol compared to when using the spin protocol. However, the purified sample using gravity protocol will be diluted as ~1.5 fold while spin protocol will gives the same volume, without any dilution.

Alternatively, a syringe-based protocol is also available on request.

Kit Components and Caution (Cat#: Exo-A300, Exo-B300, or Exo-C300)

- The mini, midi, or maxi exosome purification columns with pre-equilibrated resin (**Table 1**)
- Adapters for midi and maxi columns are included.
- Pre-equilibrated resin in PBS containing trace sodium azide. The column resin should be re-equilibrated with PBS or your desired buffer prior to use.
- The maximum sample volume for each type of column is indicated in **Table 1**.
- The viscosity of a sample can limit the sample separation performance. High viscosity causes instability during the separation and an irregular flow pattern reducing resolution.
- The performance of exosome purification column is sensitive to centrifuge force and time duration.
- These columns are designed for either spin- or gravity-based purification procedure. Both have almost equal performance.

Table 1. Basic characteristics of exosome purification column.

Product Name	Resin Volume, Column Number	Collection Tube	Spin Protocol (100g)		Gravity Protocol	
			Sample Volume	Eluate Volume	Sample Volume	Eluate Volume
ExoA300 (Mini)	0.6ml/column, 20 columns	2 ml	60 ~100µl	60 ~100µl	60 ~100µl	150µl
ExoB300 (Midi)	3.5 ml/column, 6 columns	15 ml /Adapter	150~500µl	150~500µl	150~500µl	750µl
ExoC300 (Maxi)	8.3 ml/column, 2 columns	50 ml /Adapter	500~1,500µl	500~1,500µl	500~1,500µl	2,100µl

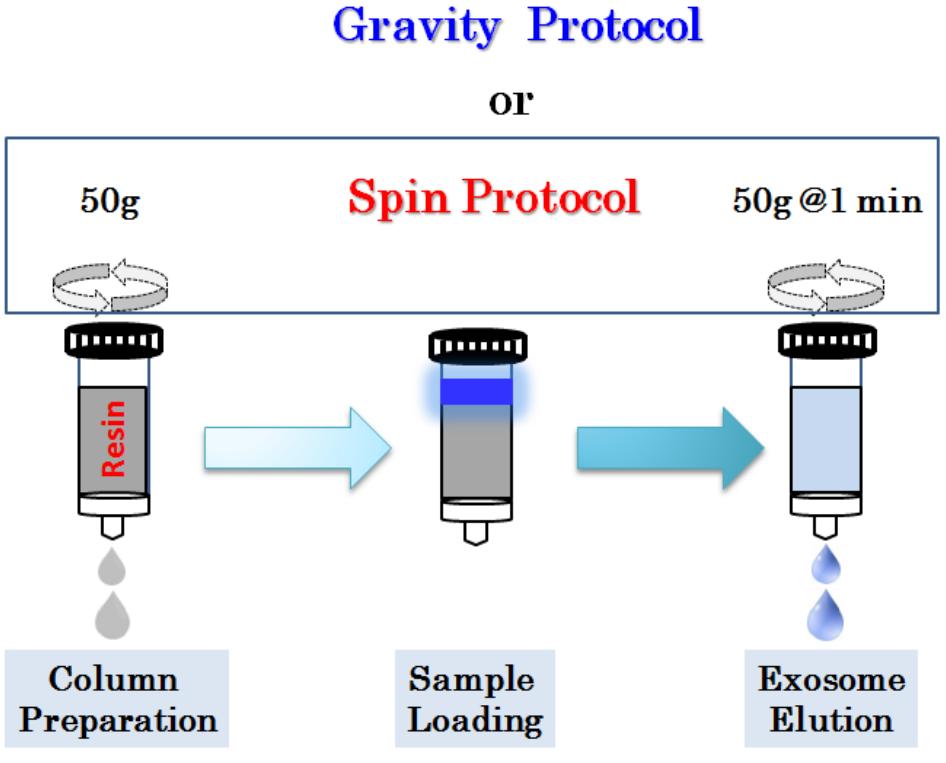

Fig.1. Chart flow of exosome purification using spin or gravity protocol.

Gravity Protocol

1. Exosome purification column preparation

- Inverting columns several times or vortexing briefly
- Remove the top and bottom cap carefully and slowly. Pinch it tightly so as air can enter the top of the column as it is being removed and reduce vacuum on the resin.
- Cut the sealed end of the column at notch or twist off bottom closure (min column)
- Put the column into a 2 ml, 15 ml or 50 ml collection tube (the midi and maxi columns need **adapter**), see Table 1.

2. Column equilibration

- Fill up the column with equilibration buffer and allow the equilibration buffer to enter the resin completely. At this point, you can add your desired buffer.
- Repeat 3 times and discard the flow-through.
- Fill up the column a fourth time with equilibration buffer
- Discard the flow-through.

3. Sample application

- Add sample slowly in the middle of the resin bed.
- Maximum sample volume for different types of columns was described in Table 1.
- If sample volume is out of the range suggested in **Table 1**,
  - For larger sample volume, use multiple columns per sample.
  - For less samples volume, allow the sample to enter the resin completely and then add desired volume of buffer so that the total volume of sample and buffer added would be optimal volume.
- Allow the sample to enter resin bed completely before any addition of buffer for elution.
- Discard the flow-through void volume, which does not contain vesicles. The void volume for mini, midi, or maxi columns is around 100 µl (2~3 drops), 0.5 ml and 1.0 ml, respectively.

4. Elution

- Place the exosome purification column into a new collection tube.
- Elute with the different volume of buffer.
- Collect the eluate which contains exosome. Typically, eluate volume of mini, midi, maxi column is around 150 µl, 0.75 ml and 1.5 ml, respectively.
- If the concentrated exosome is desired, the purified exosome can be further concentrated using Exosome Isolation Kit (Cat#: **ExoCC50**).

Spin Protocol

1. Exosome purification column preparation

- Inverting columns several times or vortexing briefly
- Remove the top and bottom cap carefully and slowly. Pinch it tightly so as air can enter the top of the column as it is being removed and reduce vacuum on the resin.
- Cut the sealed end of the column at notch or twist off bottom closure (min column)
- Put the column into a 2 ml, 15 ml or 50 ml collection tube (the midi and maxi columns need adapter, while mini column needs micro-centrifuge), see Table 1.

2. Column equilibration

- Fill up the column with equilibration buffer and allow the equilibration buffer to enter the resin completely. At this point, you can add your desired buffer.
- Repeat 3 times and discard the flow-through.
- Fill up the column a fourth time with equilibration buffer
- and spin down at 50~60 x *g* for 15-30 seconds.
- Discard the flow-through.

3. Sample application

- Add sample slowly in the middle of the resin bed.
- Maximum sample volume for different types of columns was described in Table 1.
- If sample volume is out of the range suggested in **Table 1**,
  - For larger sample volume, use multiple columns per sample.
  - For less samples volume, allow the sample to enter the resin completely and then add desired volume of buffer so that the total volume of sample and buffer added would be optimal volume.
- Allow the sample to enter resin bed completely before any addition of buffer for elution.

4. Elution

- Place the exosome purification column into a new collection tube.
- Elute by centrifugation 50~60 x *g* for 1 minutes.
- Collect the eluate. Typically, the eluate volume of mini, midi, or maxi column is around 100 µl, 0.5 ml or 1.0 ml, respectively. However, it totally depends on your initial sample volume.

Application

1. The purified exosomes will be suitable for most of applications, such as protein mass spectrometer, RNA isolation (Do *not use classical TRIZOL reagent for miRNA isolation!*), ELISA and western blot, *in vitro* loading of RNAs, and *in vivo* animal study.
2. We recommend to use the fresh isolated exosomes immediately. Otherwise please store at 4˚C for overnight, or freeze at -20˚C or -80˚C for longer periods. Note that repeated thaw and freeze cycles can lead to some loss of exosomes.