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Quick-Start Protocol

exoEasy Maxi Kit

This protocol is for purifying exosomes and other extracellular vesicles (EVs) from 0.2–4 mL of serum or plasma, or up to 8 mL of cerebrospinal fluid (CSF), or up to 16 mL of urine, or from up to 32 mL of cell culture supernatant. The binding capacity for cell culture supernatant varies strongly depending on cell type and culture conditions.

The exoEasy Maxi Kit (cat. no. 76064) is shipped at ambient temperature. Store all components dry at room temperature (15–25°C). All kit components are stable for at least 9 months upon arrival under these conditions.

Further information

- exoEasy Maxi Kit *Handbook*: **www.qiagen.com/HB-1953**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- For isolation of EVs from cell culture supernatant, either serum-free culture medium has to be used, or medium prepared with vesicle-free serum.
- All steps should be performed at room temperature (15–25°C). Carry out the protocol steps quickly but carefully.
- Centrifugation of exoEasy spin columns should be performed in a swinging bucket rotor.
- Buffer XE is produced sterile, but without preservative to prevent bacterial growth. Take appropriate measures to keep the buffer sterile after use (storing frozen in single-use aliquots, for example).

Procedure

1. It is recommended to use only pre-filtered plasma or cell culture supernatant. Supernatants should be filtered to exclude particles larger than 0.8 µm (e.g., using Sartorius® Minisart® NML (cat. no. 16592) or Merck Millipore® Millex®-AA (cat. no. SLAA033SB) syringe filters).

   Important: For cell culture supernatants, filtering should be performed prior to freezing of samples.

2. Add 1 volume buffer XBP to 1 volume of sample. Mix well by gently inverting the tube 5 times. Let the mixture warm up to room temperature.
3. Add the sample/XBP mix onto the exoEasy spin column and centrifuge at 500 x *g* for 1 min. Discard the flow-through and place the column back into the same collection tube.
4. Add 10 mL buffer XWP and centrifuge at 5000 x *g* for 5 min to remove residual buffer from the column. Discard the flow-through together with the collection tube.

   Note: It is possible to reduce the centrifugation speed from 5000 x *g* down to a minimum force of 3000 x *g* without loss of performance.

   Optional: Repeat step 4 to further reduce nonspecifically bound materials (e.g. free proteins). Reuse collection tube from step 4.
5. Transfer the spin column to a fresh collection tube.
6. Add 250–400 µL Buffer XE to the membrane and incubate for 1 min. Centrifuge at 500 x *g* for 5 min to collect the eluate.

   Note: Using less than 250 µL elution buffer will result in incomplete elution. Eluates can be concentrated e.g., by ultrafiltration. If an ultrafiltration step will be performed, eluting in 1–2 mL is recommended.

   Optional: Re-apply the eluate to the exoEasy spin column membrane and incubate for 1 min. Centrifuge at 5000 x *g* for 5 min to collect the eluate and transfer to an appropriate tube (not supplied).

Document Revision History

Date	Changes
05/2015	Initial release
01/2023	Updated protocol to include an additional wash step. Layout and editorial changes.

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