Quick-Start Protocol May 2015

# exoEasy Maxi Kit

## Notes before starting

- This protocol is for purifying exosomes and other extracellular vesicles (EVs) from 0.2–4 ml of serum or plasma, or from up to 16 ml of cell culture supernatant. The binding capacity for cell culture supernatant varies strongly depending on cell type and culture conditions.
- 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 (e.g., store frozen in single-use aliquots).

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 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.
5. Transfer the spin column to a fresh collection tube.
6. Add 400 µl – 1 ml 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 400 µl elution buffer will result in incomplete elution. Eluates can be concentrated e.g., using ultrafiltration. If an ultrafiltration step will be performed, eluting in 1–2 ml is recommended.
7. 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).



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