Quick-Start Protocol October 2017

miRCURY® Exosome Cell/Urine/CSF Kit

The miRCURY Exosome Cell/Urine/CSF Kit (cat. no. 76743) is shipped at ambient temperature. All solutions should be kept tightly sealed and stored protected from light at 2–8°C. These reagents should remain stable for at least 6 months in their unopened containers.

We recommend using the starting volumes below, but this protocol is scalable for any starting volume from 1–10 ml.

- Urine samples: 2–10 ml
- CSF samples: 1 ml
- Cell-conditioned media: 1–10 ml

Further information

- miRCURY Exosome Kits Handbook: **www.qiagen.com/HB-2434**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Important points before starting

- Make sure that the sample collection, treatment and storage up to this point have been uniform among the individual samples.
- Any purification is highly dependent on the amount of starting material. The standard protocol is flexible for isolating exosomes from 1–10 ml starting material, simply by adjusting the amount of Precipitation Buffer. Add 0.4 volumes Precipitation Buffer for every 1 volume sample.

- To obtain a cell-free specimen, centrifuge at 3000 *x g* (~2000 rpm) for 5–10 min to pellet cells and debris. Transfer the supernatant, as the fraction of interest, into a new tube prior to storage or use. We recommend centrifuging previously spun, frozen samples once more after thawing to remove cryoprecipitates. Refer to the *miRCURY Exosome Kits Handbook* for more details.
- Extraction of exosomes may also precipitate a considerable amount of microvesicles. If this is of concern, we recommend reducing the microvesicle fraction through filtration using a 0.2–0.22 µm syringe or spin-top filter (not provided) before starting the isolation protocol. Refer to Appendix A in the *miRCURY Exosome Kits Handbook* for more details.

Things to do before starting

- Ensure that the microcentrifuge to be used is at 20°C.

Procedure

Follow the instructions according to your starting volume:

- For 1 ml, follow the instructions marked with a triangle (▲).
- For 10 ml, follow the instructions marked with a circle (●).
- For other starting volumes, adapt the volumes of Precipitation Buffer B and Resuspension Buffer accordingly.

1. Prepare or thaw frozen samples on ice or at 4°C.

   Note: Centrifuge the samples to remove cells and debris, as described in "Important points before starting".

2. Transfer ▲1.0 ml sample to a new 2 ml microcentrifuge tube or ●10 ml sample to a new 15 ml conical tube.
3. Gently mix Precipitation Buffer B. Add ▲400 µl or ●4 ml Precipitation Buffer B to the sample. Close the tube and vortex to mix thoroughly.

   Note: Do not vortex Precipitation Buffer B, as this will cause foaming and pipetting difficulty.

4. Incubate for 60 min at 2–8°C.

   Note: This precipitation step can be extended to overnight, if needed.

5. Centrifuge at ▲10,000 *x g* or ●3200 *x g* for 30 min at 20°C.

6. Remove the supernatant and discard or save for separate analysis, if needed.

   Note: Some exosome pellets are not visible (e.g., exosome pellets from 3 ml urine samples). The location of the pellet in the tube depends on the type of rotor used. A fixed-angle rotor will smear the pellet along the side of the tube, while a swinging-bucket rotor will pellet the exomes at the bottom of the tube.

7. Centrifuge the pellet again for 5 s, and remove any residual supernatant.

8. To Resuspend the pellet for storage or exosome analysis, add 100 µl Resuspension Buffer to the tube containing the pellet, and resuspend by vortexing for 15 s.

   Note: The purified exosome sample may be stored at 2–8°C for 2 days or can be stored at –15 to –30°C. To minimize the risk of RNase contamination, we recommend proceeding directly with further downstream sample processing.

   For RNA purification, refer to the *miRCURY Exosome Kits Handbook* for details.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.