Quick-Start Protocol October 2017

miRCURY® Exosome Serum/Plasma Kit

The miRCURY Exosome Serum/Plasma Kit (cat. no. 76603) 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. Thrombin is shipped lyophilized at ambient temperature and is stable for at least 6 months after resuspension when stored at 2–8°C.

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 0.2–1.4 ml starting material, simply by adjusting the amount of Precipitation Buffer and Resuspension Buffer without any additional steps. Add 0.4 volumes Precipitation Buffer 1 for every 1 volume serum/plasma.
- To obtain a cell-free specimen with low platelet content, centrifuge at 3000 x g (~2000 rpm) for 5–10 min to pellet cells, debris and platelets. 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 Appendix A in the *miRCURY Exosome Kits Handbook* for more details.

- If downstream RNA expression profiling is planned, we do not recommend using hemolyzed samples. Even traces of red blood cells in serum or plasma will affect the RNA profile. Instead, we recommend using serum or citrate/EDTA plasma and discourage using heparin plasma. RNA isolated from heparin plasma can reduce PCR performance.
- 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 details.

Things to do before starting

- If you are processing plasma samples, ensure that the Thrombin has been resuspended in the Thrombin Buffer. To prepare a 500 U/ml working solution, add 400 µl Thrombin Buffer to the vial of lyophilized Thrombin. Incubate at room temperature (15–25°C) for 1 min. Gently swirl the tube to redissolve completely, but avoid vigorous mixing, as mechanical shearing could affect the enzyme quality. Aliquot and store at –20°C for later use. Avoid repeated freeze–thaw cycles. The working solution is stable for at least 6 months.
- Ensure that the microcentrifuge to be used is at 20°C.

Procedure for serum samples

Follow the instructions according to your starting volume:

- For 0.5 ml serum, follow the instructions marked with a triangle (▲).
- For 1.4 ml serum, follow the instructions marked with a circle (●).
- For other starting volumes, adapt the volumes of Precipitation Buffer A and Resuspension Buffer accordingly.

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

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

2. Transfer ▲0.5 ml or ●1.4 ml serum into a new 2 ml microcentrifuge tube.
3. Gently mix Precipitation Buffer A. Add ▲200 µl or ●560 µl Precipitation Buffer A to the serum sample. Close the tube and vortex for 5 s.

   Note: Do not vortex Precipitation Buffer A, 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 1500 *x g* for 30 min at 20°C.
6. Remove the supernatant and discard or save for separate analysis, if needed.
7. Briefly centrifuge the pellet again, and remove any residual supernatant.
8. Add ▲270 µl or ●240 µl Resuspension Buffer to the tube containing the pellet, and resuspend by vortexing. This will result in a final volume of ~300 µl.

   Note: If you are resuspending multiple samples, we recommend using a thermal mixer for 2 ml microcentrifuge tubes at 1400 rpm for 15 min at 20°C.

   Note: The purified exosome sample may be stored at 2–8°C for up to 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.

Procedure for plasma samples

Follow the instructions according to your starting volume:

- For 0.6 ml plasma, follow the instructions marked with a triangle (▲).
- For 1.7 ml plasma, follow the instructions marked with a circle (●).
- For other starting volumes, adapt the volumes of Thrombin, Precipitation Buffer A and Resuspension Buffer accordingly.

1. Prepare or thaw frozen samples on ice or at 4°C.
2. Transfer ▲0.6 ml or ●1.7 ml plasma to a 2.0 ml microcentrifuge tube.

3. Add ▲6 µl or ●17 µl Thrombin (stock concentration of 500 U/mL) to the sample. Mix and incubate for 5 min at room temperature (15–25°C).
4. Centrifuge at 10,000 *x g* for 5 min.
5. Transfer ▲0.5 ml or ●1.4 ml supernatant into a new 2 ml microcentrifuge tube.
6. Gently mix Precipitation Buffer A. Add ▲200 µl or ●560 µl Precipitation Buffer A to the supernatant. Close the tube cap and vortex for 5 s.
   Note: Do not vortex Precipitation Buffer A, as this will cause foaming and pipetting difficulty.
7. Incubate for 60 min at 4°C.
   Note: This precipitation step can be extended to overnight, if needed.
8. Centrifuge at 500 *x g* for 5 min at 20°C.
9. Remove the supernatant and discard or save for separate analysis, if needed.
10. Briefly centrifuge the pellet again, and remove any residual supernatant.
11. Add ▲270 µl or ●240 µl Resuspension Buffer to the tube containing the pellet, and resuspend by vortexing. This will result in a final volume of ~300 µl.
    Note: If you are resuspending multiple samples, we recommend using a thermal mixer for 2 ml microcentrifuge tubes at 1400 rpm for 15 min at 20°C.
    Note: The purified exosome sample may be stored at 2–8°C for up to 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. Refer to the *miRCURY Exosome Kits Handbook* for RNA purification recommendations.

Scan QR code for handbook.

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

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