January 2024

miRCURY® Exosome Kits Handbook

For enrichment of exosomes and other extracellular vesicles from serum/plasma or cells/urine/CSF

Sample to Insight

Contents

Kit Contents

The miRCURY Exosome Isolation Kit – Serum and Plasma consists of the components described below:

miRCURY Exosome Serum/Plasma Kit **Catalog no.** **Number of preps**	**76603** **16 x 1.5 or 50 x 0.25 mL**
Precipitation Buffer A	10 mL
Resuspension Buffer	10 mL
Thrombin (lyophilized)*	250 U
Thrombin Buffer*	500 µL
Quick-Start Protocol	1

* Preparation of a working solution required. See notes prior to use.

The miRCURY Exosome Isolation Kit – Cells, Urine and CSF consists of the components described below:

miRCURY Exosome Cell/Urine/CSF Kit **Catalog no.** **Number of preps**	**76743** **100 x 1 mL or 24 x 5 mL or 12 x 10 mL**
Precipitation Buffer B	2 x 25 mL
Resuspension Buffer	10 mL
Quick-Start Protocol	1

Shipping and Storage

The miRCURY Exosome Serum/Plasma Kit (cat. no. 76603) and the miRCURY Exosome Cell/Urine/CSF Kit (cat. no. 76743) are shipped at ambient temperature. All solutions should be kept tightly sealed and stored protected from light at 2–8°C. Upon arrival, these reagents should remain stable for at least 6 months in their unopened containers. Thrombin is shipped lyophilized at ambient temperature and should be stored at 2–8°C upon arrival. Thrombin will be stable for at least 6 months after resuspension when stored at 2–8°C.

Intended Use

The miRCURY Exosome Serum/Plasma Kit and the miRCURY Exosome Cell/Urine/CSF Kit are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety**, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Body fluids such as serum or plasma of all human and animal subjects are considered potentially infectious. Take all necessary precautions recommended by the appropriate authorities in the country of use when working with body fluids.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of miRCURY Exosome Serum/Plasma Kit and miRCURY Exosome Cell/Urine/CSF Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The miRCURY Exosome Kits provide a rapid method for the isolation and purification of exosomes and other extracellular vesicles (EVs) from serum/plasma or biofluids such as cerebrospinal fluid (CSF), urine, or cell culture media.

Exosomes are cell-derived membranous particles with a size of 20–120 nm, approximately the same size as viruses but considerably smaller than microvesicles. Exosomes are excreted from cells into the surrounding media and can be found in many, if not all, body fluids. Their proposed role as intercellular hormone-like messenger together with their stability as carrier of proteins and RNA makes them of great interest in the search for biomarkers for a variety of biological questions.

Principle and procedure

This extracellular vesicle isolation procedure is based on the capture of water molecules, which otherwise form the hydrate envelope of particles in suspension. Mixing the starting sample with the Precipitation Buffer diminishes the hydration of the subcellular particles and allows precipitation of even particles smaller than 100 nm with a low-speed centrifugation step.

This handbook includes two main protocols for various starting samples and amounts – Protocol: Enrichment of Exosomes from Serum or Plasma on page 11 and Protocol: Enrichment of Exosomes from Cells, Urine or CSF on page 16. These procedures are designed to allow subsequent miRNA isolation from the extracellular vesicle subpopulations. In addition, increased sample amounts can be processed with little inhibition effect on downstream PCR analysis.

For plasma and serum samples, the fractionation begins with pelleting of residual cells, debris, platelets, apoptotic bodies, etc. A more complete removal of platelets or large microvesicles can be achieved by starting with a simple filtration of the sample through 0.2 µm filter spin

columns (not provided). Such a step has been shown beneficial for certain biofluid biomarker signatures.* See Appendix A on page 22 for more detailed recommendations for serum and plasma collection, separation, and storage.

The protocols consist of 5 simple steps (see also Figure 1):

1. Thawing samples on ice or at 4°C.
2. Pelleting of dead cells and debris (as well as platelets and fibrin for plasma and serum).
3. Mixing of the sample with the Precipitation Buffer and incubation at 4°C.
4. Pelleting of the exosomal fraction.
5. Resuspension for further processing or characterization.

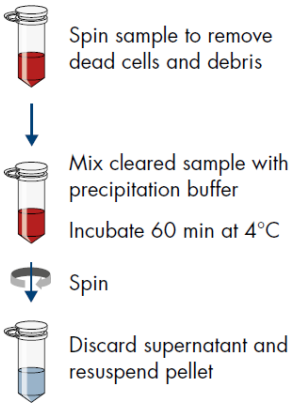

Figure 1. Protocol overview of the miRCURY Exosome Kits.

* Cheng, H.H. et al. (2013) Plasma processing conditions substantially influence circulating microRNA biomarker levels. PLoS One. 8(6):e64795.

Table 1. Specifications for miRCURY Exosome Kits

Description	miRCURY Exosome Serum/Plasma Kit	miRCURY Exosome Cell/Urine/CSF Kit
Particles precipitated	Exosomes, microvesicles and to some degree, larger protein complexes	Exosomes, microvesicles and to some degree, larger protein complexes
Maximum volume of starting material supported	1400 µL	10 mL
Recommended volume of starting material	500–1400 µL	1–10 mL
Resuspension volume for working with intact exosomes	≥300 µL Resuspension Buffer	≥100 µL Resuspension Buffer
Starting volume for RNA isolation using the miRNeasy Serum/Plasma Kit	Up to 200 µL resuspended EVs	Up to 200 µL resuspended EVs
Resuspension volume when proceeding with RNA isolation using the miRNeasy Micro Kit	700 µL QIAzol®	700 µL QIAzol
Time to complete 10 isolations	<120 min	<120 min

Equipment and Reagents to be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

The following supplies are also required for both miRCURY Exosome Kits:

- Pipette and RNase-free pipet tips
- 1.5 mL or 2–2.2 mL microcentrifuge tubes, depending on the sample volume used
- Microcentrifuge with rotor for 2 mL tubes for centrifugation at 20°C
- Disposable gloves
- Vortexer or multi-tube vortex shaker
- Refrigerator

If using the miRCURY Exosome Serum/Plasma Kit:

- miRNeasy® Micro Kit (cat. no. 217084) or miRNeasy Serum/Plasma Kit (cat. no. 217184) for downstream RNA isolation

If using the miRCURY Exosome Cell/Urine/CSF Kit:

- Centrifuge with rotor for 15 mL conical tubes for centrifugation at 20°C, if processing larger sample volumes
- miRNeasy Micro Kit (cat. no. 217084) or miRNeasy Serum/Plasma Kit (cat. no. 217184) for downstream RNA isolation

Important Notes

Transportation and storage of biofluid samples

When storing and transporting biofluid samples intended for exosome isolation, it is recommended to keep samples at 2–8°C or –15°C to –30°C. If samples are frozen, they should be centrifuged prior to freezing. Store or ship cell-free supernatants to avoid contamination with cellular components that may be released from cells during freezing and thawing. Vortex thoroughly upon thawing.

Downstream RNA isolation

If you are using the miRCURY Exosome Kits and plan to purify RNA from the isolated EVs, we recommend using the miRNeasy Micro Kit (cat. no. 217084) or the miRNeasy Serum/Plasma Kit (cat. no. 217184) for optimal results.

If the isolated EVs are used for RNA isolation only, we recommend resuspending them directly in 700 µL QIAzol lysis buffer and following the miRNeasy Micro protocol for RNA isolation.

If some of the isolated EVs will be used for other applications, we recommend resuspending them in the resuspension buffer provided with the miRCURY kits and using up to 200 µL of the resuspended EVs for subsequent RNA isolation using the miRNeasy Serum/Plasma Kit.

Any RNA spike-ins should be added during the RNA isolation procedure to prevent degradation of the spike-ins.

Protocol: Enrichment of Exosomes from Serum or Plasma

This protocol describes the purification of EVs from serum or plasma samples using the miRCURY Exosome Serum/Plasma Kit (cat. no. 76603). The procedures are scalable for any serum or plasma starting volume from 0.2–1.4 mL, depending on the need for downstream analysis. We generally recommend a starting volume of 0.5–1.0 mL.

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 A for every 1 volume of serum/plasma.
- The exosome composition from serum and plasma may give different results in downstream analyses, depending on the treatment of the sample prior to RNA isolation. Ensure that sample acquisition conditions and pretreatment are controlled and defined. To obtain a cell-free specimen with low platelet content, centrifuge at 3000 x *g* 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: Recommendations for Serum and Plasma Collection, Separation, and Storage.
- 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.

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 500 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 –15°C to – 30°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 the serum or thaw the frozen serum samples on ice or at 4°C.
2. Transfer ▲0.5 mL or ●1.4 mL supernatant into a new ▲1.5 mL or ● 2 mL microcentrifuge tube.
3. Gently mix the Precipitation Buffer A. Add ▲200 µL or ● 560 µL Precipitation Buffer A to the supernatant. Close the tube and vortex for 5 s.

   Note: This protocol describes the amount of Precipitation Buffer A for either 0.5 mL or 1.4 mL serum. Adapt the volume of Precipitation Buffer A according to your starting volume.
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 completely and discard or save for separate analysis, if needed.
7. Briefly centrifuge the pellet again and remove any residual supernatant.

   For RNA isolation directly from the EV pellets, skip step 8 below and follow the miRNeasy Micro Kit protocol for Purification of Total RNA, including miRNA, from Animal Cells, starting with step 2 (page 20 of the *miRNeasy Micro Kit Handbook*).
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: This protocol describes the amount of Resuspension Buffer for either 0.5 mL or 1.4 mL serum. Adapt the volume of Resuspension Buffer according to your starting volume.

   Note: If you are processing multiple samples, we recommend using a vortex shaker for 2 mL microcentrifuge tubes for 5–15 min at room temperature (15–25°C).

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

Note: For RNA purification from the resuspended pellet, we recommend using the miRNeasy Serum/Plasma Kit for optimal results. Refer to the *miRNeasy Serum/Plasma Kit Handbook* for details.

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).

   Note: This protocol describes the amount of Thrombin for either 0.5 mL or 1.4 mL plasma. Adapt the volume of Thrombin according to your starting volume.
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 the 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.

   Note: This protocol describes the amount of Precipitation Buffer A for either 0.5 mL or 1.4 mL plasma. Adapt the volume of Precipitation Buffer A according to your starting volume.
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 completely and discard or save for separate analysis, if needed.
10. Briefly centrifuge the pellet again, and remove any residual supernatant.

    For RNA isolation directly from the EV pellets, skip step 11 and follow the miRNeasy Micro Kit protocol for Purification of Total RNA, including miRNA, from Animal Cells, starting with step 2 (page 20 of the *miRNeasy Micro Kit Handbook*).

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: This protocol describes the amount of Resuspension Buffer for either 0.5 mL or 1.4 mL plasma. Adapt the volume of Resuspension Buffer according to your starting volume.

    Note: If you are processing multiple samples, we recommend using a vortex shaker for 2 mL microcentrifuge tubes for 5–15 min at room temperature (15–25°C).

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

    Note: For RNA purification from the resuspended pellet, we recommend using the miRNeasy Serum/Plasma Kit for optimal results. Refer to the miRNeasy Serum/Plasma Kit Handbook for details.

Protocol: Enrichment of Exosomes from Cells, Urine, or CSF

This protocol describes the purification of EVs from cell culture supernatants (conditioned medium), urine, or CSF using the miRCURY Exosome Cell/Urine/CSF Kit (cat. no. 76743). The procedure is scalable for any starting volume from 1–10 mL. We recommend the following starting volumes:

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

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 without any additional steps. Add 0.4 volumes Precipitation Buffer for every 1 volume sample.
- The exosome composition from biofluids may give different results in downstream analysis depending on the treatment of the sample prior to RNA isolation. Ensure that sample acquisition conditions and pretreatment are controlled and defined. For example, centrifuge samples at 3000 x *g* for 5–10 min to pellet the cell debris. Transfer the supernatant as the fraction of interest into a new tube prior to use or storage.
- Extraction of urine exosomes may also precipitate a considerable amount of microvesicles. If this is of concern, we recommend reducing the microvesicle fraction through a simple filtration step using a 0.2–0.22 µm syringe or spin-top filter (not provided) before starting with the actual isolation protocol.

Things to do before starting

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

Procedure for cells, urine, or CSF

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 the frozen samples and put on ice or at 4°C.

   Note: Centrifuge 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 supernatant. Close the tube and vortex for 5 s to mix thoroughly.

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

4. Incubate for 60 min at 4°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.

   For RNA isolation directly from the EV pellets, skip step 8 below and follow the miRNeasy Micro Kit protocol for Purification of Total RNA, including miRNA, from Animal Cells, starting with step 2 (page 20 of the *miRNeasy Micro Kit Handbook*).

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 a few days or can be stored at –15°C to –30°C prior to RNA isolation. To minimize the risk of RNase contamination, we recommend proceeding directly with further downstream sample processing.

   Note: For RNA purification from the resuspended pellet, we recommend using the miRNeasy Serum/Plasma Kit for optimal results. Refer to the *miRNeasy Serum/Plasma Kit Handbook* for details.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx** (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
Both kits	
Precipitates in buffers	In case you observe precipitate in one of the buffers, briefly heat the buffer to 45°C and mix until the precipitates disappear.
miRCURY Exosome Isolation Kit – Serum and Plasma	
No pellet visible	Serum and plasma should result in relatively large pellets. In case there is no visible pellet, check that Precipitation Buffer A was added and that the incubation at 4°C was carried out for at least 60 min.
Pellet not completely resuspended	If the plasma sample was centrifuged at more than 500 x *g*, the pellet will be difficult to resuspend. We have observed good RNA isolation from partially resuspended pellets after addition of QIAzol lysis buffer from the miRNeasy Serum/Plasma Kit. Work swiftly when resuspending the pellet and proceeding with RNA purification. Do not allow the pellet to stand longer than necessary.
Platelet/microvesicle removal	Pretreatment of serum and plasma samples should be uniform. Platelets should be separated from plasma with initial density centrifugation after sample acquisition, to obtain platelet-poor plasma. In addition, potentially present microvesicles (and platelets) can be removed by a filtration step through a 0.2–0.22 µm filter (not provided) before the actual precipitation step.
Low RNA yield from exosomes	RNA content of EVs is very low. Typically, no more than 5 ng of RNA are obtained from 1 mL of serum or plasma from healthy donors.
miRCURY Exosome Isolation Kit – Cells, Urine, and CSF	
No pellet visible	Some biofluids will only result in a relatively faint, sometimes invisible pellet. Lack of a clearly visible pellet does not mean a failure in your precipitation. Make sure that Precipitation Buffer B was added and that the incubation time at 4°C was carried out for at least 60 min.
Small starting volumes	If working with volumes below 1.0 mL, reduce the volume of Precipitation Buffer B accordingly. We do not recommend using starting volumes below 500 µL. Working with smaller volumes will reduce yield.

	Comments and suggestions
Exosomes from breastmilk	Centrifuge the milk sample at 5000 x *g* for 30 min to remove fat. After removing the top layer, centrifuge again at 5000 x *g* for another 30 min to remove fat residue and cell debris. Finally, centrifuge at 10,000 x *g* for 30 min. Then mix 1 volume of supernatant with 0.4 volumes of Precipitation Buffer B and incubate overnight at 4°C. Centrifuge for 30 min at 3200 x *g* at room temperature (15–25°C). Discard both phases and resuspend the pellet.

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

Appendix A: Recommendations for Serum and Plasma Collection, Separation, and Storage

To specifically isolate circulating cell-free DNA and RNA from serum and plasma, we recommend following these protocols, which include an initial low g-force centrifugation step to separate cells from plasma or serum followed by a higher g-force centrifugation or filtration step to remove all remaining cellular debris. The latter centrifugation/filtration step significantly reduces the amount of cellular or genomic DNA and RNA in the sample. Because of the much higher abundance of DNA and RNA in cells (several orders of magnitude), even small amounts of cellular debris can have a very significant effect on analysis of cell-free nucleic acid. The sooner after blood collection this removal of cellular materials is performed, the lower the risk of additional background from blood cell-derived nucleic acid released *in vitro*. Use of gel barrier tubes generally results in fewer residual cells.

The speed at which the second centrifugation step is performed will influence the recovery of different types of nucleic acid. Centrifugation at medium speed (e.g., 3000 x *g*) will effectively remove cellular material, including thrombocyte fragments and apoptotic bodies. Centrifugation at higher speed (e.g., 16,000 x *g*) may in addition remove intact chromatin from ruptured blood cells, but may also remove larger extracellular vesicles that may contain cell-free nucleic acid (especially mRNA).

Syringe filters with 0.8 µm pore size (e.g., Sartorius® Minisart® NML (cat. no. 16592) or Millipore® Millex®-AA (cat. no. SLAA033SB)) remove remaining cell fragments and debris strictly based on size, irrespective of density. These filters have dead volumes of around 100–200 µL.

Procedure: plasma separation and storage

1. Collect whole blood in BD Vacutainer® Venous Blood Collection Tubes (cat. no. 367525) containing EDTA (or other anticoagulant, e.g., citrate). Store tubes at room temperature (15–25°C) or 2–8°C and process within 1 hour.

   Note: Do not use heparin-containing blood collection tubes, as this anticoagulant can interfere with downstream assays, such as RT-PCR.

2. Centrifuge blood samples in primary blood collection tubes for 10 min at 1900 x *g* (3000 rpm) and 4°C using a swinging bucket rotor.

3. Carefully transfer the upper (yellow) plasma phase to a new tube (with conical bottom) without disturbing the intermediate buffy coat layer (containing white blood cells and platelets). Normally up to 4–5 mL plasma can be obtained from 10 mL of whole blood.

   Note: Carryover of white blood cells and platelets from the buffy coat layer is the most likely source of cellular miRNA/RNA contamination in plasma.

4. Centrifuge plasma samples in conical tubes for 15 min at 3000 x *g* (or 10 min at 16,000 x *g* – see above) and 4°C or pass through a 0.8 µm filter (see recommendations above).

5. This will remove additional cellular nucleic acids attached to cell debris.

6. Carefully transfer cleared supernatant to a new tube without disturbing the pellet, which forms a smear along the outer side or bottom of the centrifugation tube.

7. Store at 2–8°C until further processing, if plasma will be used for nucleic acid purification on the same day. For longer storage, keep plasma frozen in aliquots at –65°C to –90°C.

   Before using frozen plasma for nucleic acid purification, thaw it at room temperature (15–25°C).

   Optional: To remove cryoprecipitates, centrifuge the thawed plasma samples for 5 min at 3000 x *g* and 4°C or pass through a 0.8 µm filter. Transfer the supernatant to a new tube, and begin the nucleic acid purification protocol.

Procedure: serum separation and storage

1. Collect whole blood in a primary blood collection tube with or without clot activator, but without anticoagulants such as EDTA or citrate (e.g., Sarstedt S-Monovette® Serum-Gel 9 mL tubes, cat. no. 02.1388). For complete clotting, leave tubes at room temperature (15–25°C) for 10 min to 1 h.

   Note: Tubes with clot activator can be processed after 10 min clotting time, while tubes without clot activator should be stored for at least 30 min at room temperature to allow clotting to take place.

2. Centrifuge tubes for 10 min at 1900 x *g* (3000 rpm) and 4°C using a swinging bucket rotor.

   Note: If using Sarstedt S-Monovette Serum-Gel 9mL tubes, a gel bed will form between the upper serum phase and the lower cellular phase, facilitating recovery of serum.

3. Carefully transfer the upper (yellow) serum phase to a new tube (with conical bottom) without disturbing the pellet containing cellular material. Normally up to 3–5 mL serum can be obtained from 10 mL of whole blood.

   Note: Prevent transfer of cellular material from the lower phase.

   Note: Serum can be used for cell-free nucleic acid purification at this stage. However, an additional filtration or centrifugation will remove additional cellular debris and minimize contamination acids by gDNA and RNA derived from damaged blood cells.

4. Centrifuge serum samples in conical tubes for 15 min at 3000 x *g* (or 10 min at 16,000 x *g* – see above) and 4°C or pass through a 0.8 µm filter (see recommendations above). This will remove additional cellular nucleic acids attached to cell debris.

5. Carefully transfer cleared supernatant to a new tube without disturbing the pellet, which forms a smear along the outer side of the centrifugation tube.

6. Store at 2–8°C until further processing, if serum will be used for nucleic acid purification on the same day. For longer storage, keep serum frozen in aliquots at –65°C to –90°C.

   Before using frozen serum for nucleic acid purification, thaw at room temperature (15–25°C).

Optional: To remove cryoprecipitates, centrifuge the thawed serum samples for 5 min at 3000 x *g* and 4°C or pass through a 0.8 µm filter. Transfer supernatant to a new tube, and begin nucleic acid purification protocol.

Ordering Information

Product	Contents	Cat. no.
miRCURY Exosome Serum/Plasma Kit	For 50 x 0.25 mL or 16 x 1.5 mL preps: Precipitation Buffer A, Resuspension Buffer, Thrombin, Thrombin Buffer	76603
miRCURY Exosome Cell/Urine/CSF Kit	For 100 x 1 mL, 24 x 5 mL or 12 x 10 mL preps: Precipitation Buffer B, Resuspension Buffer	76743
Related Products		
miRNeasy Serum/ Plasma Kit (50)	For 50 total RNA preps: 50 RNeasy MinElute Spin Columns, Collection Tubes (1.5 mL and 2 mL), QIAzol Lysis Reagent, RNase-free Reagents and Buffers	217184
RNeasy Micro Kit (50)	50 RNeasy MinElute Spin Columns, Collection Tubes (1.5 mL and 2 mL), RNase-free DNASE I Carrier RNA, RNase-free Reagents and Buffers	74004

Document Revision History

Revision	Description
12/2017	Initial release.
10/2023	Updated Thrombin amount (lyophilized) from 200 U to 250 U in “Kit Contents”. Also updated the Thrombin Buffer amount from 400 µL to 500 µL on page 12.
01/2024	Updated the Kit Contents for miRCURY Exosome Cell/Urine/CSF Kit

Limited License Agreement for miRCURY Exosome Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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