miRCURY™ Exosome Isolation Kit - Cells, urine and CSF

Instruction manual v1.3

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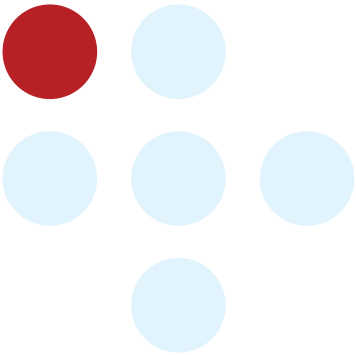

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Table of Contents

Product summary

miRCURY™ Exosome Isolation Kit – Cells, urine and CSF

miRCURY™ Exosome Isolation Kit – Cells, urine and CSF consists of the components described in Table 1.

Table 1.

Kit Components (12-100 isolations)	Amount supplied
Precipitation Buffer B	2 x25 mL
Resuspension Buffer	10 mL

Additional required material

For All Protocols

- Benchtop microcentrifuge
- Swing bucket centrifuge
- Pipette (+ RNase free tips)
- Vortexer / multi-vial vortex shaker
- Refrigerator
- miRCURY™ RNA Isolation Kit - Cell & Plant (for downstream RNA isolation)

Product description

miRCURY™ Exosome Isolation Kit – Cells, urine and CSF provides a rapid method for the isolation and purification of exosomes from biofluids like Cerebrospinal fluid (CSF), urine or cell culture media.

> Exosomes are cell derived membranous particles with a size of 20 to 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 ideal in the search for biomarkers for a variety of biological questions.

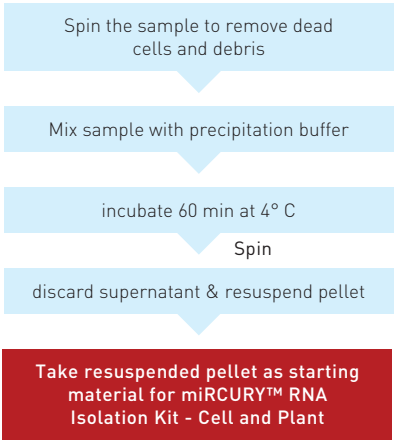
The purification is based on capturing of water molecules which otherwise form the hydrate envelope of particles in suspension. By mixing the sample with the precipitation buffer the hydration of the particles will be diminished. This allows precipitation of the subcellular particles below 100 nm with a low speed centrifugation step.

The protocols are validated to allow subsequent RNA isolation using the miRCURY™ RNA Isolation Kit – Cell and Plant and improve the quality of the obtained miRNA signature according to the exosomal subpopulation analyzed and the increased amount of sample that can be used.

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

1. Thawing samples on ice or at 4°C
2. Pelleting of dead cells / debris
3. Mixing of the sample with the precipitation buffer and incubation at 4°C
4. Pelleting of the exosomal fraction
5. Resuspension or lysis for further processing or characterization

Figure 1. Protocol overview of the miRCURY™ Exosome Isolation Kit, followed by RNA isolation using the miRCURY™ RNA Isolation Kit – Cell and Plant (prod no. 300110).



Important note - Cautions

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn and standard safety precautions are followed when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). Body fluids of all human and animal subjects are considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with body fluids.

Table 2.

Kit Specifications	
Particles precipitated	Exosomes, microvesicles and to some degree larger protein complexes
Maximum volume of starting material supported	10 mL
Recommended volume of starting material	1000 µL - 10 mL
Resuspension volume to work with intact exosomes	100 µL Resuspension Buffer
Resuspension volume when proceeding with RNA isolation	350 µL Lysis Solution
Time to complete 10 exosome isolations	<120 min

Storage and product stability

All reagents should be kept tightly sealed and stored protected from light at 4°C. These reagents should remain stable for at least 6 months in their unopened containers.

Important note - Transportation/storage of biofluid samples

When storing and transporting biofluid samples intended for exosome isolation, it is recommended to use 4° C or -20° C. If samples are frozen they should be centrifuged prior to freezing, store or ship the cell free supernatant to avoid contamination of the samples with cellular components that otherwise may be released from cells during freeze thawing. Vortex thoroughly upon thawing.

Downstream RNA isolation

If extracting RNA from the isolated exosomes, please use miRCURY™ RNA Isolation Kit - Cell & Plant for optimal result. RNA isolation protocol found in appendix page 15. Any RNA spike-ins should be added in the RNA isolation procedure (see page 15) to prevent RNA degradation of the spike-ins.

Protocol & notes

Exosome isolation from cell culture media, urine or CSF

Notes Prior to Use

- Make sure that the sample collection and treatment and storage up to this point has been as uniform as possible amongst the individual samples.
- Any preparation is highly dependent on the amount of starting material. The standard protocol is flexible to extract exosomes from 1,000 µL up to 10 mL starting material without any additional steps simply by adjusting the amounts of precipitation buffer.
- For RNA isolation we recommend to resuspend the exosome pellet directly in 350 µL Lysis Solution provided with the miRCURY™ RNA Isolation Kit - Cell & Plant. When intact exosomes are required for your downstream procedure use 100 µL of the resuspension buffer to dissolve the exosome pellet. Both volumes are adapted to fit into Cell & Plant RNA Isolation Kit protocol section 1I, see appendix in this manual.
- The exosome composition from biofluids may give different results in downstream analysis depending on the treatment of the body fluid prior to RNA isolation. Please ensure that sample acquisition conditions and specimen pretreatment are controlled and defined, e.g. centrifuge with 3000 x g (~2,000 RPM) for 5 to 10 minutes in order to pellet cell debris. Transfer the supernatant as fraction of interest into a new vial prior to use or storage.
- Extraction of urine exosomes may also precipitate a considerable amount of microvesicles. When this is of concern we recommend to reduce the microvesicle fraction through a simple filtration step using a 0.2/0.22µm syringe or spin top filter (not provided) before starting with the actual isolation protocol.
- For downstream RT-qPCR we recommend not to work with hemolysed samples as this may affect the results. For further information please also read the miRCURY™ microRNA QC PCR panel manual:
  www.exiqon.com/ls/Documents/Scientific/QC-PCR-Panel-Manual.pdf

Two protocols are available depending on starting volume, but protocols are scalable and can be used for any starting volume from 1-10 mL:

Section A. Protocol for exosome enrichment from 10 mL sample, please go to page 8
Section B. Protocol for exosome enrichment from 1.0 mL sample, please go to page 10

Recomended starting volume:	
Urine samples	2-5 mL
CSF samples	1 mL
Cell conditioned media	1-10 mL

Section A. Exosome precipitation from 10 mL sample

Notes prior to use

Before getting started please ensure that the centrifuge is run at room temperature. Thaw samples on ice or at 4°C. Vortex Precipitation Buffer B prior to use.

Step 1	Spin 10 mL sample for 5 minutes at 3,200 x g in a 15 mL conical tube to remove cell debris.
Step 2	Transfer supernatant into a new 15 mL conical tube.
Step 3	Add 4 mL Precipitation Buffer B and vortex for 5 seconds to mix.
Step 4	Incubate for 60 minutes at 4°C *
Step 5	Spin for 30 minutes at 3,200 x g at 20°C
Step 6	Remove supernatant completely and discard (or save for separate analysis). Respin the pellet 5 seconds and remove residual supernatant. Continue directly with Step 7a for RNA isolation or 7b for storage or exosome analysis.

Step 7a	For RNA extraction resuspend pellet by vortexing 15 seconds in 350 µL Lysis Solution (miRCURY™ RNA Isolation Kit - Cell & Plant, section 1I step two, see appendix in this manual).
Step 8a	Continue RNA extraction with the resuspended pellet using miRCURY™ RNA Isolation Kit - Cell & Plant protocol section 1I step three and ongoing, see appendix in this manual.

Step 7b	Resuspend pellet by vortexing 15 seconds in 100 µL Resuspension Buffer to obtain intact exosomes.
Step 8b Storage	The purified exosome sample may be stored at 4°C for a few days or can be stored at -20°C prior to RNA isolation. For RNA isolation from this point follow section 1I of the miRCURY™ RNA Isolation Kit - Cell & Plant protocol starting with step one (see appendix in this manual). In order to maximize yield and minimize the risk of RNase contamination we recommend proceeding directly with further downstream sample processing (see Step 7a).

*Precipitation time can be extended e.g. overnight.

Section B. Exosome precipitation from 1.0 mL sample

Notes prior to use

Before getting started please ensure that the centrifuge is run at room temperature. Thaw samples on ice or at 4°C. Vortex Precipitation Buffer B prior to use.

Step 1	Spin 1.1 mL sample for 5 minutes at 10,000 x g to remove cell debris.
Step 2	Transfer 1.0 mL of supernatant into a new 2 mL reaction vial.
Step 3	Add 400 µL Precipitation Buffer B invert tube several times and vortex for 5 seconds to mix thoroughly.
Step 4	Incubate for 60 minutes at 4°C.*
Step 5	spin for 30 minutes at 10,000 x g at 20°C.
Step 6	Remove supernatant completely and discard (or save for separate analysis). Respin the pellet 5 seconds and remove residual supernatant. Continue directly with Step 7a for RNA isolation or 7b for storage or exosome analysis.

Step 7a	For RNA extraction resuspend pellet by vortexing 15 seconds in 350 µL Lysis Solution (miRCURY™ RNA Isolation Kit - Cell & Plant, section 1I step two, see appendix in this manual).
Step 8a	Continue RNA extraction with the resuspended pellet using miRCURY™ RNA Isolation Kit - Cell & Plant protocol section 1I step three and ongoing, see appendix in this manual.

Step 7b	Resuspend pellet by vortexing in 100 µL Resuspension Buffer to obtain intact exosomes. For multiple samples use a 2 mL reaction vial vortex shaker for 15 seconds.
Step 8b Storage	The purified exosome sample may be stored at 4°C for a few days or can be stored at -20°C prior to RNA isolation. For RNA isolation from this point follow section 1I of the miRCURY™ RNA Isolation Kit - Cell & Plant protocol starting with step one (see appendix in this manual). In order to maximize yield and minimize the risk of RNase contamination we recommend proceeding directly with further downstream sample processing (see Step 7a).

*Precipitation time can be extended e.g. overnight.

Tips and Troubleshooting

No pellet visible

Some biofluids will only give relatively faint, sometimes invisible pellet. The lack of a clearly visible pellet does not mean a failure in your precipitation.
Please make sure that the precipitation buffer has been added and that the incubation time at 4°C has been kept for >60 minutes.

Crystal formation in buffers

In case you observe crystal formation in one of the buffers briefly heat the buffer to 45°C and mix until the crystals disappear.

Small starting volumes

If working with smaller volumes than 1.0 mL, use protocol in section B and reduce Precipitation Buffer volume accordingly. It is not recommended to work with less than 500 µL starting volume. Working with smaller volumes will reduce yield.

Exosomes from breastmilk

Spin the milk at 5,000 x g for 30 minutes to remove fat. After removing the top layer, spin again at 5,000 x g for another 30 minutes to remove fat residue and cell debris. Finally spin at 10,000 x g for 30 minutes. Mix X mL supernatant with 0.4 x X mL Precipitation Buffer B and incubate overnight at 4°C. Spin 30 minutes at 3,200 x g at room temperature and discard both phases and dissolve pellet.

Related products

Exiqon offers a specialized RNA isolation kit enabling preparation from larger amounts of human/animal tissues as well as cells and plants and downstream application products to analyze and verify the expression, function and spatial distribution of miRNAs:

miRCURY™ RNA Isolation Kit – Cell and Plant
Total RNA preparations from cultured animal cells, small tissue samples, blood, bacteria, yeast, fungi, bacteria, plants or Exosomes from Cells urine CSF or similar.

miRCURY™ RNA Isolation Kit – Biofluids
Small RNA preparations from serum or plasma samples or from exosomes from serum or plasma. Suitable for miRCURY LNA™ Universal RT microRNA PCR.

miRCURY™ RNA Isolation Kit -Tissue
Get high quality total RNA suitable for miRCURY LNA™ Universal RT microRNA PCR or miRCURY LNA™ microRNA Array analysis in as little as 20 minutes. Total RNA preparations from 15-30 mg animal/human tissue.

miRCURY LNA™ Universal RT microRNA PCR
Exiqon's microRNA qPCR system offers the best available combination of performance and ease-of-use on the microRNA real-time PCR market. The combination of a Universal RT reaction and LNA™-enhanced PCR primers results in unmatched sensitivity and specificity. The Ready-to-use microRNA PCR panels enable fast and easy microRNA expression profiling. Take advantage of the tailored Universal RT microRNA PCR spike in kit to monitor the performance of your PCR.

miRCURY LNA™ microRNA Detection
For in situ hybridization and northern blotting of all annotated microRNAs. miRCURY LNA™ microRNA ISH Buffer Set (FFPE).

miRCURY LNA™ microRNA ISH Optimization kit (FFPE)
Complete kit with control probes and hybridization buffer for easy set up of microRNA in situ hybridization.

miRCURY LNA™ microRNA Inhibitors and Power Inhibitors

Unravel the function of microRNAs by microRNA inhibition. Sophisticated LNA™ design ensures potent inhibition of all microRNAs regardless of their GC content. Chemically modified, highly stable Power Inhibitors for unrivaled potency.

miRCURY LNA™ microRNA Inhibitor Library

For genome-wide high throughput screening of microRNA function.

Appendix

RNA preparation from exosomes (modified from section 1I from miRCURY™ RNA Isolation Kit – Cell & Plant manual).

Notes prior to use

Before getting started please ensure that ethanol has been added to the Wash Solution. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

Step 1 Prepare exosome samples	Transfer up to 100 µL of exosome suspension to an RNase-free microcentrifuge tube (not provided)
Step 2 Lysis	Add 350 µL of Lysis Solution. Lyse exosomes by vortexing for 15 seconds. Ensure that the mixture becomes transparent before proceeding to the next step.
Step 3 Add Etanol	Add 200 µL of 95 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.
Step 4 Bind RNA to column	Assemble a column with one of the provided collection tubes. Apply the lysate with the ethanol onto the column and centrifuge for 1 minute at 14,000 x g. **Note:** Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional 1 minute. Discard the flowthrough and reassemble the spin column with its collection tube

Step 5 Wash	Apply 400 µL of Wash Solution to the column and centrifuge for 1 minute at 14,000 x g. Discard the flowthrough and reassemble the spin column with its collection tube. Repeat washing the column 2 more times by adding another 400 µL of Wash Solution and centrifuging for 1 minute at 14,000 x g. Discard the flowthrough and reassemble the spin column with its collection tube. Spin the column for 2 minutes at 14,000 x g in order to thoroughly dry the resin. Discard the collection tube.
Step 3 RNA Elution	Place the column into a fresh 1.7 mL Elution tube provided with the kit. Add 100 µL of Elution Buffer to the column. Centrifuge for 2 minutes at 200 x g, followed by 1 minute at 14,000 x g.
Step 4 RNA Storage	The purified RNA sample may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage. Centrifuge for 2 minutes at 200 x g, followed by 1 minute at 14,000 x g

Literature citations
Please refer to miRCURY™ Exosome Isolation Kit – Cells,urine and CSF when describing a procedure for publication using this product.

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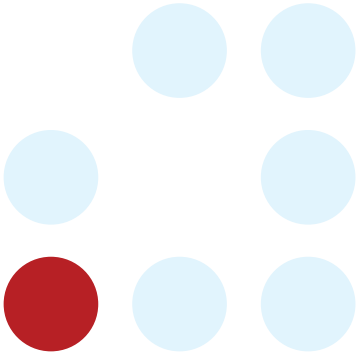

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