miRCURY™ RNA Isolation Kit - Biofluids

Instruction manual v1.7

#300112 and #300113

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EXIQON

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Product summary

miRCURY™ RNA Isolation Kit – Biofluids contents

The miRCURY™ RNA Isolation Kit – Biofluids consists of the components described in Table 1.

Table 1.

Kit Components	50 isolations	10 isolations
Lysis Solution BF	13 mL	13 mL
Protein Precipitation Solution BF	5 mL	5 mL
Reaction buffer for rDNase	7 mL	7 mL
rDNase, RNase-free (lyophilized)	1 vial *	1 vial *
Wash Solution 1 BF	10 mL	10 mL
Wash Solution 2 BF (Concentrate)	25 mL **	6 mL ***
RNase-Free Water	13 mL	13 mL
microRNA Mini Spin Columns BF	50	10
Collection Tubes (1.5 mL)	50	10
Collection Tubes (2 mL)	50	10
Collection Tubes with lid (2 mL)	50	10

* For preparation of working solution see "Protocol & notes". ** Add 100 mL 99% ethanol. *** Add 24 mL 99% ethanol.
Note: In order to streamline manufacturing we have changed bottles from square to round and volumes have been modified slightly. Content is unchanged.

Additional required materials, not supplied

- Benchtop microcentrifuge
- Pipette (+ RNase-free tips)
- Vortexer
- 99% ethanol
- Isopropanol
- Proteinase K (optional)
- MS2 RNA (Highly recommended, for increased reproducibility) , Roche, cat. no. 10165948001
- RNA Spike-In Kit (#203203), optional for quality control of extraction efficiency

Product description

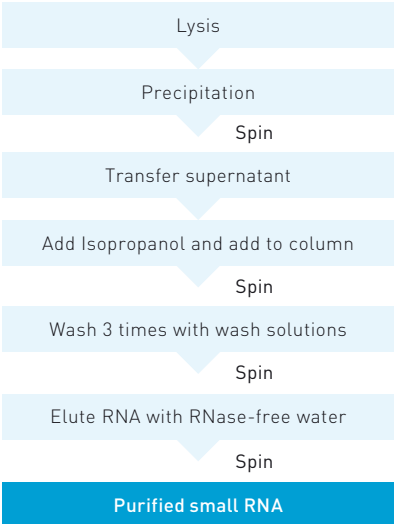
Exiqon's miRCURY™ RNA Isolation Kit – Biofluids provides a rapid method for the isolation and purification of RNA from serum, plasma and other biofluids like cerebrospinal fluid or urine (for kit specifications see Table 2). The kit can be used for the purification of all RNAs smaller than 1000 nt, from mRNA and tRNA down to microRNA and small interfering RNA (siRNA). The purification is based on spin column chromatography using a proprietary resin as the separation matrix. Small RNAs are separated from other cellular components such as proteins, without the use of phenol or chloroform in 40-70 minutes.

The kit can be used to isolate high quality RNA in amounts sufficient for qPCR analysis using the miRCURY LNA™ Universal RT microRNA PCR system.

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

1. Biofluid components are lysed with the provided Lysis Solution
2. Proteins are precipitated with the provided Protein Precipitation Solution
3. Isopropanol is added to the collected supernatant and the solution is loaded to the column with an option for an on-column DNase digestion
4. The solution is washed with Wash Solutions 1 and 2
5. The RNA is eluted with RNase free water

Figure 1. Protocol overview of the miRCURY™ RNA Isolation Kit – Biofluids.



In the first part of the RNA isolation process, membranized particles/cells are lysed using the provided Lysis Solution (Figure 1). Proteins are precipitated using the precipitation solution and isopropanol is then added to the transferred supernatant. This solution is loaded onto a spin-column. The resin binds RNA in a manner that depends on ionic concentrations. Thus only the RNA will bind to the column, while the residual proteins will be removed in the flow-through or retained on the top of the resin. The bound RNA is then washed with the provided Wash Solution I and II in order to remove any remaining impurities, and the purified small RNA is eluted with RNase free water.

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. Guanidine Thiocyanate contained in the Lysis Solution BF and in Wash Solution 1 BF is an irritant. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). Biofluids like serum or plasma 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 biofluids.

Table 2.

Kit Specifications	
Column Binding Capacity	200 µg
Maximum Column Loading Volume	800 µL
Size of RNA Purified	all RNA < 1000 nt
Maximum volume of Starting Material supported	900 µL*
Recommended volume of Starting Material	200 µL
Elution volume	20-50 µL
Time to complete 10 Purifications	40 min (70 min with optional DNase digestion)

* requires volume adjustment of Lysis Solution, Protein Precipitation Solution and Isopropanol. Sample should be loaded onto the column in aliquots. Each aliquot should be spun down before the next one is added.

Storage and product stability

- Lyophilized rDNase should be stored at -20 °C upon arrival. Storage of lyophilized rDNase at room temperature is not critical.

All other solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 6 months in their unopened containers.

Protocol overview

Before starting the experiment

Working with RNA

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. If working with RNA it is recommended as a first step to create an RNase-free environment following the precautions below.

- The RNA area should be located away from microbiological work stations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc.
- It is recommended that gloves are changed frequently to avoid contamination
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA work only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA samples, ensure that they remain on ice

Centrifugation Procedures

All centrifugation steps in this protocol are carried out in a benchtop microcentrifuge at room temperature. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. The correct rpm can be calculated using the formula:

$$\mathbf{RPM} = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

Where RCF = required gravitational acceleration (relative centrifugal force in units of g); r = radius of the rotor in cm; and RPM = the number of revolutions per minute required to achieve the necessary g-force.

Protocol & notes

General recommendations for working with biofluids

Serum/plasma

When working with serum or plasma it is generally recommended not to work with hemolyzed samples. Even traces of red blood cells in the serum or plasma may affect the microRNA profile. It is recommended to follow recommendations found in the microRNA QC PCR manual: http://www.exiqon.com/ls/Documents/Scientific/QC-PCR-Panel-Manual.pdf.

After collection of serum or plasma it is important to centrifuge the sample in order to obtain cell free starting material. Centrifuge at no more than 3000 *x g* (~2,000 RPM) for 5 minutes to pellet cells. Typically we rarely see more than 200 microRNAs present in any given non-hemolyzed serum or plasma sample (special cases where more microRNAs are found may exist for certain samples).

Urine

Generally, only small amounts of microRNAs are found in urine. However certain disease states give rise to an increased microRNA level in the urine. If patients/animals have been subjected to toxicology experiments, increased amounts of microRNAs related to the injury are typically found in the urine. When profiling microRNA from urine samples it is important to ensure that the urine sample is free from cells in order to avoid contaminating the sample with microRNAs from kidney or bladder cells. We therefore recommend that the starting material should be centrifuged at no more than 3000 *x g* for 5 minutes. This should be done **prior to storage** rather than immediately before isolation.

Cerebrospinal fluid (CSF)

CSF samples can be difficult to work with due to the very low levels of microRNAs in combination with the presence of PCR inhibitors. We rarely identify more than approximately 30 microRNAs in CSF samples. However, certain disease states may affect the number of microRNAs that can be detected. When CSF samples are extracted it can be difficult to avoid contamination from adjacent tissue, so in order to obtain a cell free starting material, centrifuge at no more than 3000 *x g* for 5 minutes to pellet cells and debris.

Exosomes

We recommend to use the miRCURY™ RNA Isolation kit - Biofluids for the RNA extraction from exosomes obtained from serum or plasma. For exosomes derived from other sources (like conditioned cell culture media, CSF or urine) we recommend the use of the miRCURY ™ RNA Isolationkit - cell plant.

Other biofluid samples

A number of other biofluids can be used with the miRCURY™ RNA Isolation Kit. The yield may vary significantly depending on organism, disease state and if the subject is medicated. The input amount of these biofluids has to be empirically determined and the amount of RNA to be used for the subsequent cDNA synthesis may have to be adjusted to avoid inhibition of the RT-reaction.

Protocols for small RNA preparation from different biofluids follow on the next pages:

Section A. Standard protocol for RNA isolation, please go to page 14
Section B. RNA isolation using a vacuum manifold, please go to page 17

Note

It is important to work quickly during the whole procedure.

Notes prior to use

Amount of starting material

Lysate preparations vary based on the starting material. Please ensure that the correct procedure for preparing the lysate from your starting material is followed. However, the subsequent steps are the same in all cases. If using less than 200 µL, we recommend filling up to 200 µL with RNase-free water. The protocol can be scaled up to 300 µL starting material without any additional steps simply by adjusting the amounts of Lysis Solution, Protein Precipitation Solution and Isopropanol in the steps prior to loading the samples onto the column.

Table 3.

	Serum/plasma	Urine	CSF	Other biofluids*
Human samples	200 µL	200 µL	200 µL	200 µL
Rodent samples	50 µL**	200 µL	Not tested	50 µL**
RNA eluate input to 20µL cDNA synthesis reaction***	4 µL	4 µL	8 µL	4 µL

*We always recommend optimizing input to sample preparation and cDNA synthesis, by investigating inhibition, since amount of inhibitors and microRNA levels vary between biofluids and organisms.
** When biofluid volume is less than 200µL, we recommend filling up to 200µL with RNase-free water.
*** For subsequent analysis using the miRCURY LNA™ Universal RT microRNA PCR system.

Recommended volumes of biofluids including input to the downstream cDNA synthesis are listed in Table 3. These volumes are just guidelines, and optimum volumes should be determined in each individual case. The kit components allow extraction of RNA from up to 900 µL biofluid. After adjusting the amounts of Lysis Solution, Protein Precipitation Solution and Isopropanol, the sample is then loaded on the column in aliquots followed by a centrifugation step.

Elution volume

The recommended elution volume is 50 µL. This volume is adapted to fit into Exiqon's PCR protocols. When extracting exosomal RNA from serum or plasma we recommend to elute in 100 µL. For other applications, a more concentrated eluate may be desirable. Reducing the elution volume to the minimum of 20 µL specifically allows concentrating small RNA while larger RNA is retained on the column.

Treatment prior to RNA isolation

The RNA composition from biofluids will give different results in downstream analysis depending on the treatment of the biofluid prior to RNA isolation. Please ensure that sample acquisition conditions and specimen pre-treatment are controlled and defined, e.g. if interested in a cell free specimen centrifuge with 3000 *x g* (~2,000 RPM) for 5 minutes in order to pellet debris and cells. Transfer the supernatant into a new vial prior to use or storage.

Avoid heparin

For downstream qPCR we recommend the use of serum or citrate /EDTA plasma and discourage using Heparin plasma. RNA isolated from Heparin plasma will significantly reduce PCR performance.

Centrifugation

All centrifugation steps are carried out in a benchtop microcentrifuge at 11,000 *x g* except where noted. A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed. All centrifugation steps are performed at room temperature.

Working temperature

Ensure that all solutions are at room temperature (18-25 °C) prior to use.

Preparation of Wash Solution 2 BF

Prepare a working concentration of the Wash Solution 2 BF by adding 100 mL of >99% ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution 2 BF (or 24mL if using the 10 prep kit). The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

Preparation of rDNase solution

Prepare a working solution of rDNase by adding 3 mL rDNase Buffer to the rDNase vial. Incubate at room temperature for 1 min. Swirl gently to redissolve completely but avoid vigorous mixing to minimize mechanical shearing which could affect enzyme quality. Aliquot and store at -20°C for later use. Avoid repeated freeze thawing. The working solution is stable for at least 6 months.

Optional Proteinase K treatment

An optional Proteinase K treatment (just prior to step 2) can improve yield and quality for some low quality samples. Add Proteinase K in a final concentration of 1-3 µg/µL to the sample. Incubate 10 min at 37°C. Then proceed to step 2 of the protocol and add Lysis Solution BF.

For subsequent qPCR analysis add carrier

To minimize the technical variation between replicates in downstream PCR analysis we highly recommend adding:

- 1µg MS2 RNA or yeast tRNA (not included) per sample to the Lysis Solution BF prior to step 1 of the protocol. Vortex/Mix to ensure homogenous distribution. For other downstram applications than the miRCURY LNA™ Universal RT microRNA PCR make sure the use of carrier does not interfere with the intended use. If RNA is to be used for Next Gen Sequencing, we recommend to use an RNA free carrier like glycogen (RNA grade). Add 2 µg per sample to the Lysis solution BF prior to step 1 of the protocol. Vortex/Mix to ensure homogenous distribution.

For subsequent qPCR analysis add RNA spike-ins

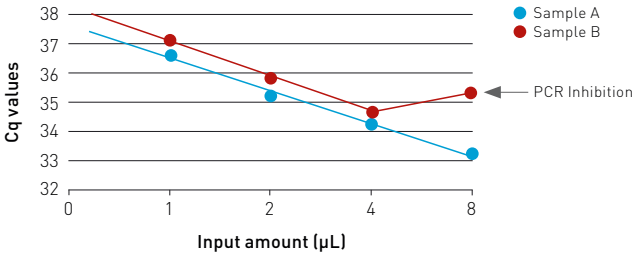
To monitor the RNA isolation via miRCURY LNA™ Universal RT microRNA PCR we recommend adding:

- 1µL Exiqon RNA spike-in mix (UniSp2, UniSp4 and UniSp5, product number #203203) per sample (not included) to the Lysis Solution BF prior to step 1 of the protocol. Vortex/Mix to ensure homogenous distribution.

Determination of yield is usually not possible by spectrophotometric reading. We therefore recommend using biofluid input amount in the PCR reaction as a measure, combined with subsequent quantification of spike-ins (if used).

In order to identify the optimum sample input as well as cDNA input, it is recommended to investigate how much RNA can be used in the cDNA without inhibiting the RT reaction. If too much RNA is added to the RT reaction, it results in non-linear behavior of microRNAs and often also a poorer call rate, due to co-extraction of inhibitors during the sample preparation. When isolating RNA from different biofluids for qPCR we recommend performing an upfront dilution curve ideally covering 4-6 data points in triplicate to verify the validity of the quantification. This dilution curve should cover the input amount in the cDNA reaction. An example of such a linearity analysis of a dilution curve is given in Figure 2. The input amount related to data point 2 would in this case allow the measurement of both samples within their linear range.

Figure 2. In order to optimize qPCR performance, it is recommended to perform a dilution curve covering different RNA inputs to the cDNA synthesis to reveal at what input volume inhibitors affect qPCR performance.



RNA yield

Since only small RNA is extracted, quantification by optical spectrophotometry or Nanodrop is not possible. We recommend that the RNA used in downstream applications are based on sample input.

Section A. Standard protocol for RNA isolation

Notes prior to use

Before getting started please ensure that isopropanol is available and that ethanol has been added to Wash Solution 2 BF and that rDNase has been resuspended in rDNase reaction buffer or that the respective rDNase aliquot is at hand. Make sure that the centrifuge is run at room temperature.

Step 1

Sample preparation

After thawing, samples should be centrifuged at 3000 *x g* for 5 min to pellet any debris and insoluble components (make sure to use excess sample in order to secure sufficient supernatant for step 2). This protocol is designed for human serum and plasma. Samples from other organisms may contain more/less inhibitors that will affect downstream applications. For rodent samples, we generally recommend using less starting material (50µL) and top up with water to 200µL.

microRNA content may vary significantly between sample types. Recommended starting volume may have to be adjusted. In order to reduce the effect of inhibitors/nucleases the starting material should be centrifuged at no more than 3000 *x g* for 5 minutes. This should be done prior to storage rather than immediately prior to isolation. Omitting this clearance step can affect subsequent PCR analysis (use excess sample in order to secure sufficient supernatant for step 2).
In order to reduce the effect of inhibitors/nucleases the starting material should be centrifuged at no more than 3000 *x g* for 5 minutes. This should be done prior to storage rather than immediately prior to isolation. Omitting this clearance step can affect PCR detection (use excess sample in order to secure sufficient supernatant for step 2).

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Step 1 Sample preparation *continued...*	**Other biofluids** Optimum input volume needs to be empirically determined to ensure high RNA yield and prevent inhibition of downstream PCR applications.
Step 2 Lysis	Transfer 200 µL supernatant from step 1 to new tube and add 60 µL Lysis solution BF Vortex for 5 sec. Incubate for 3 min at room temperature. **Recommendation:** For downstream PCR analysis, add 1 µL RNA Spike-in template mixture (miRCURY LNA™ Universal RT microRNA PCR, RNA Spike-in kit) and 1µg carrier-RNA/60µL Lysis Solution BF.
Step 3 Protein Precipitation	Add 20 µL of Protein Precipitation Solution BF. Vortex for 5 sec. Incubate for 1 min at room temperature. Centrifuge for 3 min at 11,000 *x g*.
Step 4 Transfer supernatant	Transfer the clear supernatant into a new collection tube (2 mL, with lid).
Step 5 Adjust binding conditions	Add 270 µL Isopropanol. Vortex for 5 sec.
Step 6* Load column	Place a microRNA Mini Spin Column BF in a collection tube and load sample onto the column. Incubate for 2 min at room temperature. Centrifuge for 30 sec at 11,000 *x g*. Discard flow-through and place column back into the collection tube. **Note:** if more than 300 µL sample was used repeat this step until all material is loaded.

* If DNA contamination is a problem, please perform an on-column DNase treatment as described in Appendix A.

Step 7a Wash and dry	Add 100 µL Wash Solution 1 BF to the microRNA spin column BF. Centrifuge for 30 sec at 11,000 *x g*. Discard flow-through and place column back into the collection tube.
Step 7b Wash and dry	Add 700 µL Wash Solution 2 BF to the microRNA spin column BF. Centrifuge for 30 sec at 11,000 *x g*. Discard flow-through and place column back into the collection tube.
Step 7c Wash and dry	Add 250 µL Wash Solution 2 BF to the microRNA spin column BF. Centrifuge for 2 min at 11,000 *x g* to dry the membrane completely.
Step 8 Elute	Place the microRNA spin column BF in a new collection tube (1.5 mL). Add 50 µL RNase free H_2O directly onto the membrane of the microRNA spin column BF. Incubate for 1 min at room temperature. Close the lid and centrifuge for 1 min at 11,000 *x g*. **Note:** the recommended 50 µL elution volume is ideal for use with Exiqon's Universal cDNA Synthesis Kit. However when isolating exosomal RNA we recommend to elute in 100 µl in order to maximize yield, the elution can be done in two steps eluting with half of the recommended total volume each.
Step 9 Storage	The purified RNA sample may be stored at –20°C for a few days. It is recommended to keep the samples at –70°C for long term storage.

Note

For downstream qPCR analysis we recommend using the PCR manual for serum/plasma and other biofluid samples, using the recommended cDNA input from Table 3.

Section B. RNA isolation using a vacuum manifold

Notes prior to use

This is a general protocol intended for use with commercially available vacuum manifolds. The vacuum system should enable a vacuum of between -800 to -900 mbar. In some cases additional extension tubes or adaptors may be necessary. For more specific questions, please contact the vendor of your vacuum manifold.

Lysis Solution BF and Wash Solution 1 BF contain Guanidine thiocyanate. Do not treat waste/flow through with acid/bleach. Acidic conditions will release toxic fumes.

Before getting started please ensure that isopropanol is available, that ethanol has been added to Wash Solution 2 BF and that rDNase has been resuspended in rDNase reaction buffer or that the respective rDNase aliquot is at hand. Make sure that the centrifuge is run at room temperature.

Step 1
Sample preparation

Serum/plasma
After thawing, samples should be centrifuged at 3000 *x g* for 5 min to pellet any debris and insoluble components (make sure to use excess sample in order to secure sufficient supernatant for step 2). This protocol is designed for human serum and plasma. Samples from other organisms may contain more/less inhibitors that will affect downstream applications. For rodent samples we generally recommend to using starting material (50µL) and top up with water to 200µL.

Cell free urine
microRNA content may vary significantly between sample types. Recommended starting volume may have to be adjusted. In order to reduce the effect of inhibitors/nucleases the starting material should be centrifuged at no more than 3000 *x g* for 5 minutes. This should be done prior to storage rather than immediately prior to isolation. Omitting this clearance step can affect subsequent PCR analysis (use excess sample in order to secure sufficient supernatant for step 2).

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Step 1 Sample preparation *continued...*	**Cerebrospinal fluid** In order to reduce the effect of inhibitors/nucleases the starting material should be centrifuged at no more than 3000 *x g* for 5 minutes. This should be done prior to storage rather than immediately prior to isolation. Omitting this clearance step can affect PCR detection (use excess sample in order to secure sufficient supernatant for step 2). **Other biofluids** Optimum input volume needs to be empirically determined to ensure high RNA yield and prevent inhibition of downstream PCR applications.
Step 2 Lysis	Transfer 200 µL supernatant from step 1 to new tube and add 60 µL Lysis solution BF. Vortex for 5 sec. Incubate for 3 min at room temperature. **Recommendation:** For downstream PCR analysis, add 1 µL RNA Spike-in template mixture (miRCURY LNA™ Universal RT microRNA PCR, RNA Spike-in kit) and 1µg carrier-RNA/60µL Lysis Solution BF.
Step 3 Protein Precipitation	Add 20 µL of Protein Precipitation Solution BF. Vortex for 5 sec. Incubate for 1 min at room temperature. Centrifuge for 3 min at 11,000 *x g*.
Step 4 Transfer supernatant	Transfer the clear supernatant into a new collection tube (2 mL, with lid).
Step 5 Adjust binding conditions	Add 270 µL Isopropanol. Vortex for 5 sec.

Step 6* Load column	Connect microRNA Mini Spin Column BF to the vacuum manifold allowing vacuum suction. Make sure unused manifold positions are sealed (no vacuum applied). Load sample to the column (no vacuum applied). Incubate for 2 min at room temperature. Apply vacuum. When all columns stop dripping switch off the vacuum and ventilate the vacuum device. **Note:** If more than 300 µL sample was used repeat the loading step until all material is loaded.
Step 7a Wash and dry	Add 100 µL Wash Solution 1 BF to the microRNA spin column BF (no vacuum applied). Switch on vacuum. When all columns stop dripping switch off the vacuum and ventilate the vacuum device.
Step 7b Wash and dry	Add 700 µL Wash Solution 2 BF to the microRNA spin column BF (no vacuum applied). Switch on vacuum. When all columns stop dripping switch off the vacuum and ventilate the vacuum device.
Step 7c Wash and dry	Add 250 µL Wash Solution 2 BF to the microRNA spin column BF (no vacuum applied). Switch on vacuum. When all columns stopped dripping switch off the vacuum and ventilate the vacuum device. Transfer microRNA Mini Spin Column BF to a collection tube (2mL). Centrifuge for 2 min at 11,000 *x g* to dry the membrane completely.

* If DNA contamination is a problem, please perform an on column DNase treatment as described in Appendix A.

Step 8 Elute	Place the microRNA spin column BF in a new collection tube (1.5 mL). Add 50 µL RNase-free H2O directly onto the membrane of the microRNA spin column BF. Incubate for 1 min at room temperature. Close the lid and centrifuge for 1 min at 11,000 *x g*. **Note:** the recommended 50 µL elution volume is ideal for use with Exiqon's Universal cDNA Synthesis Kit. However when isolating exosomal RNA we recommend to elute in 100 µl In order to maximize yield the elution can be done in two steps eluting with half of the recommended total volume each.
Step 9 Storage	The purified RNA sample may be stored at –20°C for a few days. It is recommended to keep the samples at –70°C for long term storage.

Note

For downstream qPCR analysis we recommend using the PCR manual for serum/plasma and other biofluid samples, using the recommended cDNA input from Table 3.

Tips and Troubleshooting

Poor RNA Recovery

Column has become clogged: In most cases this can happen when recommended amounts of starting materials were exceeded. For most biofluids this is unlikely to occur. However, because of the variety of biological samples the amount of starting material may need to be decreased below the recommended levels if the column shows signs of clogging. See also "Clogged Column" below.

An alternative elution solution was used: For maximum RNA recovery it is recommended to elute the RNA with the RNase-free water supplied with this kit.

Ethanol was not added to the Wash solution: Ensure that 100 mL of >99% ethanol is added to the supplied Wash Solution 2 BF prior to first use (or 24mL if using the 10 prep kit).

Binding condition not adjusted: Ensure that the membrane is washed in isopropanol according to step 4 in the protocol.

Low RNA content in specimen used: Different biofluids have different RNA contents, and thus the expected yield of RNA can vary greatly between different sample sources. Please check the literature to determine the expected RNA content of your starting material. Biofluids often contain limited amounts of small RNA and no DNA or larger RNA molecules. For some applications or sample sources an additional concentration step may be advantageous. Moreover note that it can be difficult to determine such low RNA concentration.

Working with urine or CSF: Urine and CSF samples contain very little microRNA. It is highly recommended to combine with miRCURY™ Exosome Isolation prior to RNA isolation. This allows for a much larger starting volume while preventing accumulation of inhibitors of the downstream qPCR.

Clogged Column

Temperature too low: Ensure that the centrifuge and solutions remain at room temperature (18-25°C) throughout the procedure. Temperatures below 15°C may result in salt precipitates that may clog the columns. If salt precipitation is present, heat the solution to 30°C until completely redissolved and let the solutions cool to room temperature before use.

Degraded RNA

RNase contamination: RNases may be introduced when working with the samples. Ensure that proper procedures are followed when working with RNA. Please refer to "Working with RNA" at the beginning of this manual.

Procedure not performed quickly enough: In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly.

Improper storage of the purified RNA: For short term storage RNA samples may be stored at –20°C for a few days. It is recommended that samples be stored at –70°C for longer term storage.

Tip!
If possible, snap freeze your RNA in liquid nitrogen before storage in the freezer.
Avoid repeated freeze/thaw-cycles by freezing aliquots of your RNA.

Enzymes used may not be RNase-free: In order to prevent possible problems with RNA degradation ensure that enzymes used upstream of the isolation process are RNase-free.

RNA does not perform well in downstream applications

Salt or Ethanol carryover: Traces of salt and ethanol from the binding step can interfere with downstream applications. Therefore, Step6 (Wash) is important for the quality of your RNA sample. To avoid remaining salts please make sure that the RNA bound to the column is washed 3 times with the provided Wash Solution and ensure that the dry spin is performed, in order to remove traces of ethanol prior to elution.

Inhibitors: Many biofluids contain inhibitors of qPCR that may to some degree be co-extracted. In order to avoid inhibition, the sample size used for the RNA isolation as well as the amount of RNA template used in the RT reaction may need to be optimized.

Genomic DNA contamination

When using **large amounts of starting material** genomic DNA contaminations can appear depending on sample type. It is possible to perform RNase-free DNase I digestion on the RNA sample during the isolation (see Appendix A) or after elution to remove genomic DNA.

Appendix A

Protocol for Optional On-Column DNA Removal

The miRCURY™ RNA Isolation Kit - Biofluids can be used to isolate small RNA with minimal amounts of genomic DNA contamination. However, an optional protocol is provided below for the removal of residual DNA that may affect sensitive downstream applications.

DNase digest after Step 6 (optional)	Add 700 µL Wash Solution 2 BF to the microRNA spin column BF. Centrifuge for 30 sec at 11,000 *x g* (or use vacuum manifold). Discard flow-through and place column back into the collection tube. Add 250 µL Wash Solution 2 BF to the microRNA spin column BF. Centrifuge for 2 min at 11,000 *x g* (or use vacuum manifold). Add 50 µL rDNase* directly onto the membrane of the microRNA spin column BF. Close lid and incubate for 15 min at room temperature. Continue with step 7.

*Dissolved in reaction buffer.

Related products

RNA Spike-In kit (203203)
RNA spike-in kit for quality control of the RNA isolation and cDNA synthesis steps of a miRCURY LNA™ Universal RT microRNA PCR experiment.

miRCURY™ Exosome Isolation Kit – Serum and plasma (300101)
Isolate Exosomes from serum or plasma for RNA isolation or other downstream applications

miRCURY™ Exosome Isolation Kit – Cells, urine and CSF(300102)
Isolate Exosomes from diverse biofluids or conditioned cell culture media for RNA isolation or other downstream applications.

miRCURY™ RNA Isolation kit -Tissue (300111)
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™ RNA Isolation kit -Cell and plant (300110)
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. The kit provides a rapid method for purification of total RNA from cultured animal cells, small tissue samples, blood, bacteria, yeast, fungi, bacteria and plants.

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 Array, microarray kit
Pre-printed miRCURY LNA™ microRNA Array microarray slides, available in pack sizes of 3, 6 and 24 for hsa, mmu & rno and other species. The kit comes complete with hybridization and wash buffers as well as synthetic spike-in microRNAs.

miRCURY LNA™ microRNA Array, ready-to-spot probe set
Ready-to-spot oligo set for direct printing of arrays, or coupling in bead-based applications (product # 208010-A).

miRCURY LNA™ microRNA Detection
For *in situ* hybridization and Northern blotting of all annotated microRNAs.

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 unrivalled potency.

miRCURY LNA™ microRNA Inhibitor Library
For genome-wide high throughput screening of microRNA function.

Further reading

- Kroh E.M. et al. (2010) Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). Methods.50:298-301

- Vickers K.C. et al. (2011) microRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nature Cell Biology 13,423–433

- Arroyo J.D. et al. (2011) Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. Proc. Natl. Acad. Sci. USA. 2108:5003-8

- Pritchard C.C. et al. (2012) Blood cell origin of circulating microRNAs: a cautionary note for cancer biomarker studies. Cancer. Prev. Res. 2012 Mar;5(3):492-7

- Eldh M. et al (2012) Importance of RNA isolation methods for analysis of exosomal RNA: Evaluation of different methods. Mol Immunology 50:278-286

- McAlexander M.A. (2013) Comparison of methods for miRNA extraction from plasma and quantitative recovery of RNA from cerebrospinal fluid. Frontiers in Genetics. doi: 10.3389/fgene.2013.00083

- Exiqon Biofluid Guidelines: http://www.exiqon.com/ls/Documents/Scientific/microRNA-serum-plasma-guidelines.pdf

Literature citations
Please refer to miRCURY™ RNA isolation kit - Biofluids when describing a procedure for publication using this product.

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