miRCURY™ RNA Isolation Kit – Cell & Plant

Instruction manual v2.4

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Product summary

miRCURY™ RNA Isolation Kit - Cell & Plant content

The miRCURY™ RNA Isolation Kit - Cell & Plant consists of the components described in Table 1.

Table 1

Kit Components (50 isolations)	Amount supplied
Lysis Solution	40 mL
RNase-Free Water	40 mL
Wash Solution	38 mL*
Elution Buffer	6 mL
Mini Spin Columns	50
Collection Tubes	100
Elution Tubes (1.7 mL)	50

* Add 90 mL 96-100% ethanol before use.

Additional required material

For All Protocols
Benchtop microcentrifuge
96 - 100% ethanol
β-mercaptoethanol (optional but recommended)

For Animal Cell Lysate Preparation
PBS (RNase-free)

For Animal Tissue Lysate Preparation
Liquid nitrogen
Mortar and pestle or rotor-stator homogenizer
70% ethanol

For Nasal or Throat Swabs Lysate Preparation
Sterile, single-use cotton swabs

For Bacterial Lysate Preparation
Lysozyme-containing TE Buffer:
- For Gram-negative bacteria, 1 mg/mL lysozyme in TE Buffer
- For Gram-positive bacteria, 3 mg/mL lysozyme in TE Buffer

For Yeast Lysate Preparation
Resuspension Buffer with Lyticase:
- 50 mM Tris pH 7.5
- 10 mM EDTA
- 1 M Sorbital
- 1 unit/µL Lyticase

For Fungi Lysate Preparation
Liquid nitrogen
Mortar and pestle
70% ethanol

For Plant Lysate Preparation
Liquid nitrogen
Mortar and pestle
70% ethanol

For Fatty tissue Lysate Preparation
Liquid nitrogen
Mortar and pestle or rotor-stator homogenizer
96-100% ethanol
Lysis Additive (Prod no. 300121)

For exosomes from conditioned cell culture media, CSF or urine
miRCURY™ Exosome Isolation Kit - Cells, urine and CSF (Prod no. 300102)
Please follow RNA isolation protocol found in Exosome isolation manual
95% ethanol

Product description

The miRCURY™ RNA Isolation Kit – Cell & Plant provides a rapid method for purification of total RNA from cultured animal cells, small tissue samples, blood, bacteria, yeast, fungi, bacteria and plants (see Table 2 for kit specifications).

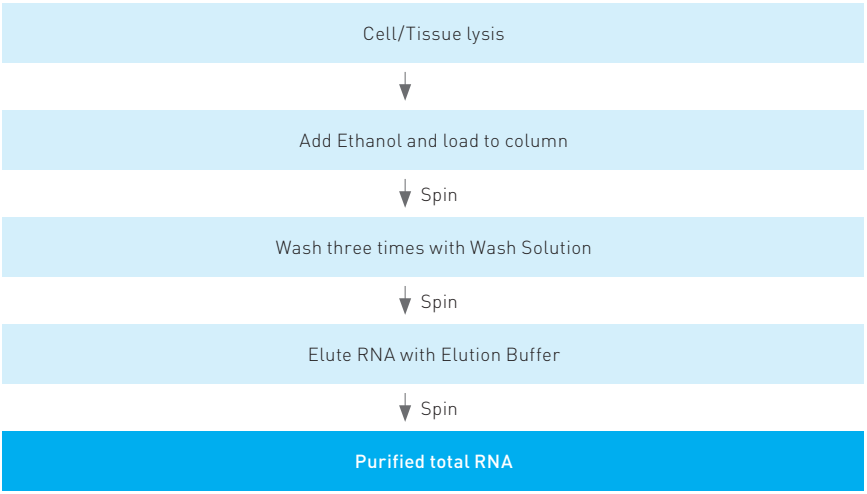
The miRCURY™ RNA Purification Kit's are based on spin column chromatography using a proprietary resin as the separation matrix. The total RNA is preferentially purified from other cell components such as proteins without the use of phenol or chloroform in an easy 20 to 50 min. protocol (depending on the sample source).

The purified total RNA is of highest quality and can be used in a number of downstream applications such as microRNA detection by miRCURY LNA™ microRNA PCR System and miRCURY LNA™ microRNA Array, mRNA expression array assays, real time PCR, Northern blotting, and RNase protection and primer extension assays.

The miRCURY™ RNA Purification Kit's include protocols optimized for each individual type of sample. The protocols consist of 4 simple steps (see Figure 1):

1. The cells and tissue are lysed with the provided Lysis Solution
2. Ethanol is added and the solution is loaded to the column
3. The RNA is washed with the included Wash Solution
4. The RNA is eluted with the included Elution Buffer

Figure 1. Protocol overview of the miRCURY™ RNA Isolation Kit- Cell & Plant.



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 buffer is an irritant. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs).
Blood or tissue of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with blood or tissue.

Kit Specifications*	
Column Binding Capacity	50 µg
Maximum Column Loading Volume	600 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material:	
Animal Cells	3 x 10^6 cells
Animal Tissues	5-10 mg (for most tissues*)
Blood	100 µL
Bacteria	1 x 10^9 cells
Yeast	1 x 10^8 cells
Fungi	50 mg
Plant Tissues	50 mg
Brain and Adipose Tissue**	10-20 mg
Exosomes from urine samples	2-5 mL
Exosomes from CSF samples	1 mL
Exosomes from cell conditioned media	1-10 mL
Time to Complete 10 Purifications	20 minutes
Average Yields:	
HeLa Cells (1 x 10^6 cells)	15 µg
E. coli (1 x 10^9 cells)	50 µg

* for isolating total RNA from larger amounts of tissue, please use Exiqon's miRCURY™ RNA Isolation Kit - Tissue (Product no. 300111).

** for isolating total RNA from brain and adipose tissue a Lysis Additive is required. The Lysis Additive can be obtained from Exiqon (Product no. 300121). See Appendix C for additonal details.

Storage and product stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.

Protocol

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 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 required for different protocol steps, are indicated in g-force. If your centrifuge has no possibility to adjust for g-force (RCF) the correct RPM can be calculated using the formula:

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

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

Section 1. Total RNA Preparation From Various Cell Types

Notes Prior to Use:

- Cell and tissue lysate preparations (section 1) are different depending on the starting material. Please ensure that the correct procedure for preparing the total RNA from your starting material is followed. However, the subsequent steps in section 2 are the same in all cases
- All centrifugation steps are carried out in a benchtop microcentrifuge. 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
- Ensure that all solutions are at room temperature prior to use to avoid precipitation in the buffer solutions
- Prepare a working concentration of the Wash Solution by adding 90 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added
- Optional: The use of β-mercaptoethanol in lysis is highly recommended for most animal tissues, particularly those known to have a high RNAse content (ex: pancreas), as well as for most plant tissues and nasal and throat swabs. It is also recommended for users who wish to isolate RNA for sensitive downstream applications. Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of Lysis Solution required. β-mercaptoethanol is toxic and should be dispensed in a fume hood. Alternatively, the Lysis Solution can be used as provided.
- It is important to work quickly during the whole procedure

Section 1A. Lysate Preparation from Cultured Animal Cells

Notes Prior to Use	The maximum recommended input of cells is 3 x 10^6. A hemocytometer can be used in conjunction with a microscope to count the number of cells. As a general guideline, a confluent 3.5 cm plate of HeLa cells will contain 10^6 cells. See Table 3 with information on cell numbers/growth areas. Cell pellets can be stored at -70°C for later use or used directly in the procedure. Determine the number of cells present before freezing. Frozen pellets should be stored for no longer than 2 weeks to ensure that the integrity of the RNA is not compromised. Frozen cell pellets should not be thawed prior to RNA isolation. Add the Lysis Solution directly to the frozen cell pellet.

Table 3

Cell-culture vessel	Growth area (cm2)*	Number of cells†
Multiwell-plates		
96-well	0.32 - 0.6	4-5 x 10^4
48-well	1	1 x 10^5
24-well	2	2.5 x 10^5
12-well	4	5 x 10^5
6-well	9.5	1 x 10^6
Dishes		
35 mm	8	1 x l0^6
60 mm	21	2.5 x l0^6
100 mm	56	7xl0^6
145 - 150 mm	145	2xlO^7
Flasks		
40-50 ml	25	3 x 10^6
250-300 ml	75	1 x 10^7
650-750 ml	162-175	2 x 10^7

*Per well, If multiwell plates are used; varies slightly depending on the supplier.

†Cell numbers are given for HeLa cells (approximate length = 15 µm), assuming confluent growth. Cell numbers will vary for different kinds of animal cells, which vary in length from 10 to 30 µm.

Step 1 Prepare cells	**Cells growing in monolayer** Aspirate media and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS.	**Cells growing in suspensions** Transfer cell suspension to an RNase-free tube (not provided) and centrifuge at no more than 200 x g for 10 minutes to pellet cells. Carefully decant the supernatant. A few µL of media may be left behind with the pellet in order to ensure that the pellet is not dislodged.
Step 2 Cell Lysis	Add 350 µL of Lysis Solution directly to culture plate. Lyse cells by gently tapping culture dish and swirling buffer around plate surface for five minutes. Transfer lysate to a microcentrifuge tube.	Add 350 µL of Lysis Solution to the pellet. Lyse cells by vortexing for 15 seconds. Ensure that the entire pellet is completely dissolved before proceeding to the next step.
Step 3 Add Ethanol	Add 200 µL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. Note: For input amounts greater than 10^6 cells, it is recommended that the lysate is passed through a 25 gauge needle attached to a syringe 5-10 times at this point, in order to shear the genomic DNA prior to loading onto the column.	

Proceed to Section 2

Section 1B. Lysate Preparation from Animal Tissues

Notes Prior to Use	miRCURY™ RNA Isolation Kit - Cell & Plant is designed for isolating total RNA from small amount of tissue sample (up to 10 mg in most cases). If a larger amount of starting material is desired, the miRCURY™ RNA Isolation Kit - Tissue should be used (product no. 300111). RNA in animal tissues is not protected after harvesting until the tissue is disrupted and homogenized in Lysis Solution. Thus, it is important that the procedure is carried out as quickly as possible, particularly the Cell Lysate Preparation steps. Fresh or frozen tissues may be used for the procedure. Tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Tissues may be stored at -70°C for several months. When isolating total RNA from frozen tissues ensure that the tissue does not thaw during weighing or prior to tissue homogenization. Tissues stored in RNA stabilization reagents such as RNAlater® are compatible with this isolation procedure. Prior to isolation, carefully remove the tissue from the storage reagent using forceps, and dry excessive liquid. The maximum recommended input of tissue varies depending on the type of tissue being used. Please refer to Table 4 below as a guideline for maximum tissue input amounts. If your tissue of interest is not included in the table below we recommend starting with an input of no more than 10 mg.

Table 4. Maximum input amounts of different tissues

Heart	5 mg
Kidney	10 mg
Liver	10 mg
Lung	10 mg
Spleen	10 mg
Brain + Adipose Tissue*	20 mg

Step 1
Prepare the tissue

Excise the tissue sample from the animal.

Determine the amount of tissue by weighing. Please refer to Table 4 for the recommended maximum input amounts of different tissues.

* For isolation of total RNA from fatty tissue including brain and adipose tissue please refer to Appendix C – modified protocol for Lysate Preparation from Fatty Tissue using Lysis Additive (Product No. 300121)

Step 2
Homogenize the tissue

Transfer the tissue into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle.

Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.

Add 600 µL of Lysis Solution to the tissue sample and continue to grind until the sample has been homogenized. Homogenize by passing the lysate 5-10 times through a 25 gauge needle attached to a syringe.

Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).

Note: A rotor-stator homogenizer is a fast and convenient alternative for homogenization of small tissue samples. Add Lysis buffer directly to the frozen tissue sample in a 2 ml tube (not provided) and homogenize.

Spin the lysate for 2 minutes at 14,000 x g to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.

Step 3
Add Ethanol

Add an equal volume of 70% ethanol (provided by the user) to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate).

Vortex to mix.

Proceed to Section 2

Section 1C. Lysate Preparation from Blood

Notes Prior to Use	Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood. It is recommended that no more than 100 µL of blood be used in order to prevent clogging of the column. We recommend the use of this kit to isolate total RNA from non-coagulating fresh blood using EDTA as the anti-coagulant.
Step 1 Prepare sample	Transfer up to 100 µL of non-coagulating blood to an RNase-free microcentrifuge tube (not provided).
Step 2 Cell lysis	Add 350 µL of Lysis Solution to the blood. Lyse cells by vortexing for 15 seconds. Ensure that mixture becomes transparent before proceeding to the next step.
Step 3 Add Ethanol	Add 200 µL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.

Proceed to Section 2

Section 1D. Lysate Preparation from Nasal or Throat Swabs

Notes Prior to Use	Body fluid of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with these samples.
Step 1 Prepare samples	Gently brush a sterile, single-use cotton swab inside the nose or mouth of the subject.
Step 2 Cell lysis	Add 600 µL of Lysis Solution to an RNase-free microcentrifuge tube (not provided). Using sterile techniques, cut the cotton tip where the nasal or throat cells were collected and place into the microcentrifuge tube containing the Lysis Solution. Close the tube. Vortex gently and incubate for 5 minutes at room temperature. Using a pipette, transfer the lysate into another RNase-free microcentrifuge tube (not provided). Note the volume of the lysate.
Step 3 Add Ethanol	Add an equal volume of 70% ethanol (provided by the user) to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.

Proceed to Section 2

Section 1E. Lysate Preparation from Bacteria

Notes Prior to Use	Prepare the appropriate lysozyme-containing TE Buffer as indicated in Table 5 (reagents provided by the user). This solution should be prepared with sterile, RNAse-free TE Buffer, and kept on ice until needed. It is recommended that no more than 10^9 bacterial cells be used in this procedure. Bacterial growth can be measured using a spectrophotometer. As a general rule, an E. coli culture containing 1 x 10^9 cells/mL has an OD_{600} of 1.0. For RNA isolation, bacteria should be harvested in log-phase growth. Bacterial pellets can be stored at -70°C for later use, or used directly in this procedure. Frozen bacterial pellets should not be thawed prior to beginning the protocol. Add the Lysozyme-containing TE Buffer directly to the frozen bacterial pellet (Step 1).
Step 1 Prepare bacteria	Pellet bacteria by centrifuging at 14,000 x g for 1 minute. Decant supernatant, and carefully remove any remaining media by aspiration. Resuspend the bacteria thoroughly in 100 µL of the appropriate lysozyme-containing TE buffer (see Table 5) by vortexing. Incubate at room temperature for the indicated time.

Table 5. Lysis of Different Bacterial Strains

Bacteria Type	Lysozyme Concentration in TE Bufffer	Incubation Time
Gram-negative	1 mg/mL	5 min
Gram-positive	3 mg/mL	10 min

Step 2
Cell lysis

Add 300 µL of Lysis Solution and vortex vigorously for at least 10 seconds.

Step 3
Add Ethanol

Add 200 µL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.

Proceed to Section 2

Section 1F. Lysate Preparation from Yeast

Notes Prior to Use	For each preparation prepare 100 µL of Lyticase-containing Resuspension Buffer (50 mM Tris, pH 7.5, 10 mM EDTA, 1M Sorbital, 0.1% β-mercaptoethanol and 1 unit/µL Lyticase)(provided by the user). This solution should be prepared with sterile, RNAse-free reagents, and kept on ice until needed. It is recommended that no more than 10^7 yeast cells or 1 mL of culture be used for this procedure. **Note:** For RNA isolation, yeast should be harvested in log-phase growth. Yeast pellets can be stored at -70°C for later use, or used directly in this procedure. Frozen yeast pellets should not be thawed prior to beginning the protocol. Add the Lyticase-containing Resuspension Buffer directly to the frozen yeast pellet (Step 1).
Step 1 Prepare yeast samples	Pellet yeast by centrifuging at 14,000 x g for 1 minute. Decant supernatant, and carefully remove any remaining media by aspiration. Resuspend the yeast thoroughly in 100 µL of Lyticase-containing Resuspension Buffer by vortexing. Incubate at 37°C for 10 minutes.

Step 2 Cell lysis	Add 300 µL of Lysis Solution Vortex vigorously for at least 10 seconds.
Step 3 Add Ethanol	Add 200 µL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.

Proceed to Section 2

Section 1G. Lysate Preparation from Fungi

Notes Prior to Use	Fresh or frozen fungi may be used for this procedure. Fungal tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Fungi may be stored at -70°C for several months. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised. It is recommended that no more than 50 mg of fungi be used for this procedure in order to prevent clogging of the column.
Step 1 Prepare samples	Determine the amount of fungi by weighing. Transfer the fungus into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the fungus thoroughly using a pestle. Note: At this stage the ground fungus may be stored at 70°C, such that the RNA purification can be performed at a later time. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.

Step 2 Cell lysis	Add 600 µL of Lysis Solution to the tissue sample and continue to grind until the sample has been homogenized. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided). Spin the lysate for 2 minutes at 14,000 x g to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.
Step 3 Add Ethanol	Add an equal volume of 70% ethanol (provided by the user) that is equivalent to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.

Proceed to Section 2

Section 1H. Lysate Preparation from Plant

Notes Prior to Use	The maximum recommended input of plant tissue is 50 mg or 5 x 10^6 plant cells. Both fresh and frozen plant samples can be used for this protocol. Samples should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
Step 1 Prepare plant tissue	Transfer ≤50 mg of plant tissue or 5 x 10^6 plant cells into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the sample into a fine powder using a pestle in liquid nitrogen. Note: If stored frozen samples are used, do not allow the samples to thaw before transferring to the liquid nitrogen. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw.
Step 2 Cell lysis	Add 600 µL of Lysis Solution to the tissue sample and continue to grind until the sample has been homogenized. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided).

Step 2 (continued)	Spin the lysate for 2 minutes at 14,000 x g to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube. Note the volume of the supernatant/lysate.
Step 3 Add Ethanol	Add an equal volume of 70% ethanol (provided by the user) that is equivalent to the lysate volume collected (100 µL of ethanol is added to every 100 µL of lysate). Vortex to mix.

Proceed to Section 2

Section 1I. Lysate Preparation from Viruses

Notes Prior to Use	For the isolation of integrated viral RNA, follow Section 1A if the starting material is cell culture, follow Section 1B if the starting material is tissue, follow Section 1C if the starting material is blood, or follow Section 1D if the starting material is a nasal or throat swab. For the isolation of RNA from free viral particles, follow this procedure below. It is recommended that no more than 100 µL of viral suspension be used in order to prevent clogging of the column.
Step 1 Prepare virus samples	Transfer up to 100 µL of viral suspension to an RNase-free microcentrifuge tube (not provided).
Step 2 Lysis	Add 350 µL of Lysis Solution. Lyse viral cells by vortexing for 15 seconds. Ensure that the mixture becomes transparent before proceeding to the next step.
Step 3 Add Ethanol	Add 200 µL of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.

Proceed to Section 2

Section 2. Total RNA Purification from All Types of samples

Note	The remaining steps of the RNA purification procedure are the same from this point forward for all the different types of samples (section 1A-1I). Keep centrifuge and buffers at room temperature.
Step 1 Bind RNA to column	Assemble a column with one of the provided collection tubes. Apply up to 600 µL of the lysate with the ethanol (from Section 1) onto the column and centrifuge for 1 minute at > 3,500 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 at 14,000 x g. Discard the flowthrough and reassemble the spin column with its collection tube. If the lysate volume exceeds 650 µL, apply the remaining lysate on the column and spin 1 minute at > 3,500 x g.

Step 1A (Optional) DNase I treatment	The miRCURY™ RNA Isolation Kit – Cell & Plant isolates total RNA with minimal amounts of genomic DNA contamination. However, if necessary an optional On-Column DNA Removal Protocol is provided in Appendix A for maximum removal of residual DNA. This step should be performed at this point in the protocol
Step 2 Wash	Apply 400 µL of Wash Solution to the column and centrifuge for 1 minute at 14,000 x g. Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute. 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 50 µL of Elution Buffer to the column. Centrifuge for 2 minutes at 200 x g, followed by 1 minute at 14,000 x g. Note the volume eluted from the column. If the entire 50 µL has not been eluted, spin the column at 14,000 x g for 1 additional minute. Note: For maximum RNA recovery you can repeat the elution step. However it is recommended to elute into a separate microcentrifuge tube to avoid dilution of the RNA sample eluted first.
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.

Tips and troubleshooting

Poor RNA Recovery

Incomplete lysis of cells or tissue

Ensure that you have used the appropriate lysis protocol and amount of Lysis Solution for your sample source and for the amount of cells or tissue you used. For yeast: Ensure that the appropriate amount of lyticase is added when making the Resuspension Buffer.

Column has become clogged

In most cases this can happen when recommended amounts of starting materials were exceeded. Nevertheless 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 clogging. See also "Clogged Column" below.

An alternative Elution Buffer was used

It is recommended that the Elution Buffer supplied with this kit be used for maximum RNA recovery.

Ethanol was not added to the lysate or Wash solution

Ensure that the appropriate amount of ethanol is added to the lysate before binding to the column and that 90 mL of 96-100% ethanol is added to the supplied Wash Solution prior to 1st use.

Residual media on Cell Culture plates and on cell, bacteria or yeast pellets

Ensure that cell monolayers are washed with the appropriate amount of PBS and that all media is removed from cell pellets through aspiration prior to the addition of the lysis solution.

Low RNA content in cells or tissues used

Different tissues and cells have different RNA contents, and thus the expected yield of RNA can vary greatly between different sample sources. Please check literature to determine the expected RNA content of your starting material.

Clogged Column

Insufficient solubilization of cells or tissues

Ensure that the appropriate amount of lysis buffer was used for the amount of cells or tissue.

Maximum number of cells or amount of tissue exceeds kit specifications

Refer to Table 2 to determine if amount of starting material falls within kit specifications.

High amounts of genomic DNA present in sample

The lysate may be passed through a 25 gauge needle attached to a syringe 5-10 times in order to shear the genomic DNA prior to loading onto the column.

Centrifuge temperature too low

Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the columns to clog.

Degraded RNA

RNase contamination

RNases may be introduced during RNA isolation. Ensure proper procedures are followed when working with RNA. Please refer to "Working with RNA" at the beginning of this user guide.

Procedure not performed quickly enough

In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly. This is especially important for the Cell Lysate Preparation Step in the Animal Tissue protocol, since the RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized in the Lysis Solution.

Improper storage or handling 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. Keep your purified RNA sample on ice.

Tip 1.

Avoid repeated freeze/thaw-cycles by freezing aliquots of your RNA. If you have to freeze your sample several times you can minimize RNA damage by snap freezing your RNA tubes in liquid nitrogen prior to storage in the freezer.

Frozen tissues or cell pellets were allowed to thaw prior to RNA isolation

Do not allow frozen tissues to thaw prior to homogenization in Lysis Solution in order to ensure that the integrity of the RNA is not compromised.

Starting material may have a high RNase content

For starting materials with high RNAase content, it is highly recommended that β-mercaptoethanol be added to the Lysis Solution.

Tip 2.

For problematic tissues (ex. Pancreas, Colon) it can be beneficial to store the tissues in RNA preserving agent (RNAlater®, Ambion) before preparation.

Enzymes used may not be RNAse-free

Ensure that the lysozyme, lyticase or DNaseI being used with this kit is RNase-free, in order to prevent possible problems with RNA degradation.

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 step 2 (Wash) in Protocol Section 2 is important for the further performance of your RNA sample.

Please make sure that the RNA bound to the column is washed 3 times in total with the provided Wash Solution and ensure that the dry spin is performed, in order to remove traces of ethanol prior to elution.

Tip 3.

If you encounter problems working with tissue samples stored in RNAlater® it is possible to rinse the tissue very briefly in RNase free water to reduce salt carryover from the RNAlater® reagent. You should continue to tissue lysis immediately to avoid degradation of RNA.

Genomic DNA contamination

When using large amounts of starting material genomic DNA contaminations can appear. For these samples it is possible to perform optional on-column DNase I digestion (see Appendix A).

Appendix A

The miRCURY™ RNA Isolation Kit - Cell & Plant isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional protocol is provided below for maximum removal of residual DNA if this is affecting your downstream applications. An RNase-free DNase I should be used for this protocol.

Optional On-Column DNA Removal

Protocol

Step 1 Prepare DNase I working solution	Prepare a working stock of 0.25 Kunitz unit/µL RNase-free DNase I solution according to the manufacturer's instructions. A 100 µL aliquot is required for each column to be treated. Alternatively, dissolve or dilute stock DNase I in a reaction buffer (40 mM Tris pH 7.0, 10 mM $MgCl_2$ and 3 mM $CaCl_2$, made RNase-free) to give a final concentration of 0.25 Kunitz unit/µL.
Step 2 Bind extracted RNA to column	Perform the appropriate Total RNA Isolation Procedure for your starting material up to and including "Binding to Column" (Section 2, Step 1).
Step 3 Wash	Apply 400 µL of Wash Solution to the column and centrifuge for 2 minutes at 14,000 x g. Discard the flowthrough. Reassemble the spin column with its collection tube.

Step 4 DNase I incubation	Apply 100 µL of the RNase-free DNase I solution prepared in Step 1 to the column. Centrifuge for 2 minutes at 200 x g. Alternatively, centrifuge for a 30 second pulse at 14, 000 x g if only a single speed centrifuge is available. Ensure that the entire DNase I solution passes through the column. Repeat the step if needed. Incubate the column assembly at 25 - 30°C for 15 minutes. During the incubation, pipette the flowthrough that is present in the collection tube back onto the top of the column
Proceed to Section 2, Step 2 (second wash)	Without any further centrifugation, proceed directly to the second wash of Section 2, Step 2 at the paragraph starting with "Repeat washing the column 2 more times...".

Appendix B

The miRCURY™ RNA Isolation Kit - Cell & Plant can also be used to clean up or concentrate RNA from samples already isolated with other methods, e. g. phenol/chloroform extraction protocols. Following the steps below the RNA is preferentially purified from protein or phenol traces that can affect downstream applications.

Clean up of phenol/chloroform extracted RNA

Protocol

Step 1 Add Lysis solution	Adjust volume to 100 µL with RNase-free Water. Add 250 µL of Lysis Solution from the Total or Tissue RNA Kit.
Step 2 Add Ethanol	Add 200 µL of 96 - 100% EtOH.
Proceed to Section 2	Proceed to "Bind RNA to column" (Section 2, Step 1).

Appendix C

For optimal isolation of RNA from tissues with high lipid content including brain and adipose tissue the following protocol using Lysis Additive should be applied. The Lysis Additive (Product No 300121) can be obtained separately at Exiqon.

Note: Two spin columns will be required for completing this protocol. Hence, the RNA Isolation kit – Cell & Plant provides suffiecient reagents and material for 25 RNA isolations using fatty tissue.

Modified Protocol for Lysate Preparation from Fatty Tissue using Lysis Additive

Table 6. Maximum input amounts of different tissues

Brain	20 mg
Adipose Tissue	20 mg
Other fatty tissues (recommended starting amount)	10 mg

Protocol. Section 1J Fatty Tissue

Notes prior to Use	Please refer to Section 1B. Lysate preparation from Animal tissue for important and detailed information.
Step 1 Prepare the tissue	Excise the tissue sample from the animal. Determine the amount of tissue by weighing. Please refer to Table 6 for the recommended maximum input amounts of different tissues.

Step 2 Homogenize the tissue	Transfer the tissue into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw. Add 300 µL of Lysis Solution to the tissue sample and continue to grind until the sample has been homogenized. Homogenize by passing the lysate 5-10 times through a 25 gauge needle attached to a syringe. Using a pipette, transfer the lysate into an RNase-free microcentrifuge tube (not provided). Note: A rotor-stator homogenizer is a fast and convenient alternative for homogenization of small tissue samples. Add Lysis buffer directly to the frozen tissue sample in a 2 ml tube (not provided) and homogenize.
Step 2B Add Lysis Additive	Add 15 µL of Lysis Additive (to be obtained separately) to the lysate. Add 300 µL RNase-Free Water. Mix by vortexing.

Step 2C Pass through column for removal of genomic DNA	Assemble a column with one of the provided collection tubes. Apply the entire lysate onto the column and centrifuge at 14,000 x g (~14,000 RPM) for 3 min. Ensure the entire lysate has passed into the collection tube by inspecting the column. Alternatively, spin for another 3 min. Transfer the flow-through containing the RNA into another RNAse-free microcentrifuge tube (not provided).
Step 3 Add Lysis Solution and Ethanol	Add 200 µL Lysis Solution and 500 µl 96-100% Ethanol (not provided) to the lysate. Mix by vortexing.
Proceed to Section 2 (starting at page 27)	Note: The DNase I treatment (Section 2, optional Step 1A) is not necessary in this protocol due to the use of Lysis Additive.

Related products

Exiqon offers a tool kit enabling new discoveries concerning the expression, function, and spatial distribution of microRNAs:

Figure 2

miRCURY RNA Isolation Kit - Tissue
Specifically designed for purification of total RNA from tissue samples.

miRCURY™ RNA Isolation Kit - Biofluids
Kit for purification of low abundant small RNAs from samples such as serum, plasma, urine and CSF.

miRCURY LNA™ microRNA Hi-Power Labeling Kit
For fluorescent labeling of microRNAs from total RNA samples ready for array hybridization.

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 for direct printing of arrays, or coupling in bead-based applications.

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™ Universal RT microRNA PCR
Exiqons 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.

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.

miRCURY LNA™ microRNA Mimics
Our sophisticated 3rd generation miRCURY LNA™ microRNA Mimics are designed to simulate naturally occurring mature microRNAs. Introduction of a microRNA mimic into cells will increase the proportion of RNA induced silencing complexes (RISC) containing the guidestrand microRNA.

miRCURY LNA™ Target Site Blockers
For inhibition of microRNA binding to a specific mRNA target.

LNA™ longRNA GapmeR
LNA™ gapmers are potent antisense oligonucleotides used for highly efficient inhibition of mRNA and lncRNA function. Designed using advanced algorithms, the RNase H-activating LNA™ gapmers offer excellent performance and high success rate.

Literature citations

Please refer to miRCURY™ RNA Isolation Kit - Cell & Plant when describing a procedure for publication using this product.

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