

# miRCURY™ RNA Isolation Kit – Tissue

## Instruction manual

Version 1.0 - December 2008  
for product# 300111

### Literature citations

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

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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.



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# Product summary

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## miRCURY™ RNA Isolation Kit - Tissue content

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

**Table 1**

<b>Kit Components (50 isolations)</b>	<b>Amount supplied</b>
Lysis Solution	40 mL
RNase-Free Water	40 mL
Proteinase K	2 vials
Wash Solution	22 mL
Elution Buffer	6 mL
Mini Spin Columns	50
Collection Tubes	100
Elution tubes (1.7 mL)	50

## Additional required material

Benchtop microcentrifuge

95 - 100% ethanol

$\beta$ -mercaptoethanol

Liquid nitrogen

Mortar and pestle or rotor-stator homogenizer

2 mL tubes (recommended for homogenization with rotor-stator)

70% ethanol

55°C incubator



## Product description

The miRCURY™ RNA Isolation Kit – Tissue provides a rapid method for purification of total RNA from all types of animal tissue samples, including fiber-rich tissues such as muscle and heart. The miRCURY™ RNA Isolation Kit – Tissue is provided with Proteinase K, which aids in the removal of the various proteins present in fiber-rich tissues including collagen, contractile proteins and connective tissues (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 30 to 50 min. protocol (depending on tissue lysis).

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, mRNA 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 also Figure 1):

1. The tissue is 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



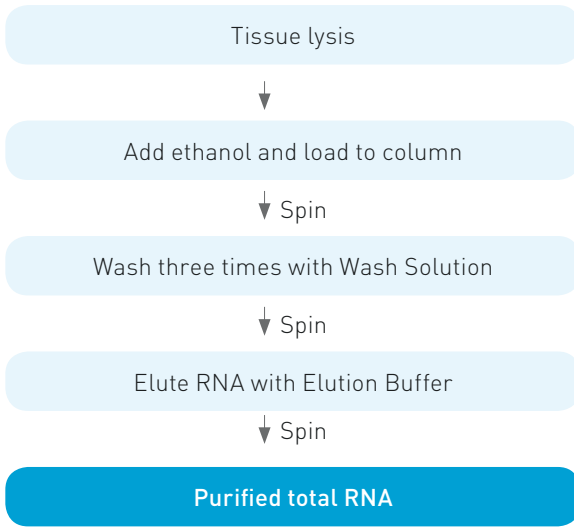
**Figure 1**

Figure 1. Protocol overview of the miRCURY™ RNA Isolation Kit- Tissue.

Table 2

<b>Kit Specifications</b>	
Column Binding Capacity	50 µg
Maximum Column Loading Volume	650 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material:	
Heart	30 mg
Kidney	15 mg
Liver	15 mg
Muscle	30 mg
Spleen	15 mg
Time to Complete 10 Purifications	50 minutes
Average Yields per 10 mg tissue	
Brain	5-20 µg
Colon	20-40 µg
Liver	15-80 µg
Muscle	5-35 µg

*For isolating total RNA from cultured animal cells, small tissue samples, blood, bacteria, yeast, fungi, bacteria and plants please use Exiqon's miRCURY™ RNA Isolation Kit - Cell & Plant (Product no. 300110).*

## 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. The Proteinase K should be stored in aliquots at -20°C upon reconstitution.



## Related products

Exiqon offers a specialized RNA isolation kit enabling preparation from cultured animal cells, small tissue samples, blood, bacteria, yeast, fungi, bacteria and plants. Exiqon also offers downstream application products to analyze and verify the expression, function and spatial distribution of microRNAs:

### **miRCURY™ RNA Isolation Kit - Cell & Plant**

Total RNA preparations from cultured animal cells, small tissue samples, blood, bacteria, yeast, fungi, bacteria and plants.

### **miRCURY LNA™ microRNA Array**

Pre-printed miRCURY LNA™ microRNA Array for expression analysis of microRNAs. Available in pack sizes of 3, 6 and 24 arrays. Available for all species.

### **miRCURY LNA™ microRNA Array, ready-to-spot probe set**

Ready-to-spot oligo set for direct printing of arrays, or coupling in beadbased applications. Available for all species.

### **miRCURY LNA™ microRNA Array, Spike-in microRNA kit**

Ten different synthetic unlabeled microRNAs in different concentrations. The spike-in microRNA kit will hybridize to corresponding capture probes on the miRCURY LNA™ microRNA Array.

### **miRCURY LNA™ microRNA Detection**

For *in situ* hybridization and Northern blotting of all annotated microRNAs.

### **miRCURY LNA™ microRNA Knockdown**

For functional inhibition of microRNA. Available as microplate libraries or as individual assays.

### **miRCURY LNA™ microRNA Real-time PCR**

Quickly and accurately determine microRNA expression using real-time PCR system.



# 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:

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

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 purification from tissue

#### Notes Prior to Use:

- 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.
- Reconstitute each of the provided Proteinase K vials in 600  $\mu$ L of molecular biology grade water or 10 mM Tris.HCl pH 7.5 (RNase-Free), aliquot into small fractions and store the unused portions at  $-20^{\circ}\text{C}$  until needed.
- Ensure that all solutions - except for Proteinase K - are at room temperature prior to use.
- Prepare a working concentration of the Wash Solution by adding 50 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution. This will give a final volume of 72 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Add 10  $\mu$ L of  $\beta$ -mercaptoethanol (provided by the user) to each 1 mL of Lysis Solution required.  $\beta$ -mercaptoethanol is toxic and should be dispensed in a fume hood.
- RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized in the Lysis Solution. Thus it is important that the procedure is carried out as quickly as possible, particularly the Cell Lysate Preparation step.
- Fresh or frozen tissues may be used for the procedure. Tissues should be flash-frozen in liquid nitrogen and transferred immediately to a  $-70^{\circ}\text{C}$  freezer for long-term storage. Tissues may be stored at  $-70^{\circ}\text{C}$  for several months. When isolating total RNA from frozen tissues ensure that the tissue does not thaw during weighing or prior to RNA preparation.



- Quick, efficient disruption and homogenization of the frozen tissue is a crucial step in RNA isolation. This can be done with mortar and pestle, using liquid nitrogen as stated in the protocol. A fast and convenient alternative is the use of a rotor-stator homogenizer. In this case add the Lysis Solution directly to the frozen tissue and homogenize without allowing the tissue to thaw.

**Note:** Flash-freezing the excised tissue in liquid nitrogen is recommended. However, if homogenization without flash-freezing is preferred, a rotor-stator homogenizer (e.g TissueRuptor, Qiagen) can be used for rapid and efficient 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 any excessive liquid.
- It is important not to exceed the recommended starting amount for each tissue. Please refer to Table 2 for the recommended maximum input amount of each tissue.
- It is important to work quickly during the whole procedure.



## Section 1. Lysate preparation from tissue

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### Step 1

Prepare the tissue

Excise the tissue sample from the animal.

Determine the amount of tissue by weighing. Please refer to Table 2 for the recommended maximum input amounts of different tissues. For tissues not included in the table, we recommend starting with an input of no more than 10 mg.



### 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  $\mu$ L of Lysis Solution to the tissue sample and continue to grind until the sample has been homogenized.

**Note:** Maximum homogenization may be achieved 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).



### Step 3

Add RNase-free water

Add 600  $\mu$ L of RNase-Free Water (provided) to the lysate. Vortex to mix.



**Step 4**

Add Proteinase K

Add 20  $\mu\text{L}$  of reconstituted Proteinase K to the lysate, and incubate at 55°C for 15 minutes. Vortex the tubes occasionally during incubation.

Spin the lysate for 1 minute at 14,000 x g to pellet any cell debris. Transfer the supernatant to another RNase-free microcentrifuge tube (not provided).

**Step 5**

Add ethanol

Add 450  $\mu\text{L}$  of 95% ethanol (provided by the user) to the lysate. Vortex to mix.



## Section 2. Total RNA preparation from tissue lysate

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### Step 1

Bind RNA to column

Assemble a column with one of the provided collection tubes.

Apply up to 650  $\mu\text{L}$  of the lysate with the ethanol (from Section 1) 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 minute.

Discard the flowthrough and reassemble the spin column with its collection tube.

If the lysate volume exceeds 650  $\mu\text{L}$ , apply the remaining lysate on the column and spin 1 minute at 14,000 x g.

**Note:** If part of the lysate has not passed into the collection tube after the last centrifugation step and the volume is less than 200  $\mu\text{L}$ , continue without additional centrifugation.



### Step 2

Wash

Apply 400  $\mu\text{L}$  of Wash Solution to the column and centrifuge at 14,000 x g for 2 minutes.

Discard the flowthrough and assemble the spin column with a new collection tube.



### Step 2A (Optional)

DNase I treatment

The miRCURY™ RNA Isolation Kit – Tissue 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 can be performed at this point in the protocol.



**Step 3**

## Wash

Apply 400  $\mu\text{L}$  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 by adding another 400  $\mu\text{L}$  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 4**

## RNA Elution

Place the column into a fresh 1.7 mL Elution tube provided with the kit.

Add 50  $\mu\text{L}$  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  $\mu\text{L}$  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 5**

## RNA Storage

The purified RNA sample may be stored at  $-20^{\circ}\text{C}$  for a few days. It is recommended that samples be placed at  $-70^{\circ}\text{C}$  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 tissue you used.

### Column has become clogged

In most cases this can happen if solubilization of tissue was insufficient or 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 50 mL of 95% ethanol is added to the supplied Wash Solution prior to first use.

### Low RNA content in cells or tissues used

Different tissues 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 the lysate is diluted with the appropriate amount of RNase-free water, and that the appropriate amount of Proteinase K is added. Also ensure that the Proteinase K treatment is performed at 55°C for the full 15 minutes. The incubation time can be increased up to 30 minutes if required.

### Maximum 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. Appendix A provides a protocol for on-column DNase I treatment.

### 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 tissue lysate preparation in “Section 1”, 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.



Tip 1

Keep your purified RNA sample on ice.

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 were allowed to thaw prior to RNA isolation**

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

### **Many tissues have a high RNase content**

For starting materials with high RNAase content, make sure  $\beta$ -mercaptoethanol has been 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**

If you perform optional DNase I treatment, make sure that the DNase I 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 and 3 (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 at once to avoid degradation of RNA.



### Genomic DNA contamination

Using large amounts of starting material in some cases 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 - Tissue 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 (not provided with the kit).

## Optional on-column DNA removal

### Protocol

#### Step 1

Prepare DNase I working solution

Prepare a working stock of 0.25 Kunitz unit/ $\mu\text{L}$  RNase-free DNase I solution according to the manufacturer's instructions.

A 100  $\mu\text{L}$  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  $\text{MgCl}_2$  and 3 mM  $\text{CaCl}_2$ , made RNase-free) to give a final concentration of 0.25 Kunitz unit/ $\mu\text{L}$ .

#### Step 2

Bind RNA to column

Perform the Total RNA Isolation procedure including "Bind RNA to column" (Section 2, Step 1).

#### Step 3

Wash

Apply 400  $\mu\text{L}$  of Wash Solution to the column and centrifuge for 2 minute at 14,000 x g. Discard the flowthrough. Reassemble the spin column with its collection tube.



**Step 4**

## DNase I incubation

Apply 100  $\mu$ L 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**

Without any further centrifugation, proceed directly to "Wash" (Section 2, Step 3).



# Appendix B

The miRCURY™ RNA Isolation Kit - Tissue 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 and ethanol

Adjust volume to 100  $\mu$ L with RNase-free Water.  
Add 250  $\mu$ L of Lysis Solution from the Total or Tissue RNA Kit



**Step 2**

Add ethanol

Add 200  $\mu$ L of 95 - 100% EtOH



**Proceed to Section 2**

Proceed to "Bind RNA to column" (Section 2, Step 1).



# References

Web page [www.exiqon.com](http://www.exiqon.com)





### **Outside North America**

Exiqon A/S · Skelstedet 16  
DK-2950 Vedbaek · Denmark  
Tech. Support +45 45 650 929  
Phone +45 45 660 888  
Fax +45 45 661 888

### **North America**

Exiqon Inc. · 14 F Gill Street  
Woburn, MA 01801 · United States  
Phone +1 781 376 4150  
Fax +1 781 376 4152  
Toll free (US) +1 888 miRCURY

[support@exiqon.com](mailto:support@exiqon.com)  
[www.exiqon.com](http://www.exiqon.com)

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