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# GenCatch™ Total RNA Miniprep Kit

## *User's Guide for*

## Total RNA Extraction From Cell, Tissue and Bacteria etc.

For Research Use Only



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# 1

Quick Start Procedure

For Experienced Users Only.

The following flow chart is only good as a quick reminder for key steps and buffers to be used. Procedure varies depending on different type of samples. First time users are strongly recommended to read through the detailed instruction protocol in section 4.

Before you start:

WARNING: strong acids and oxidants (bleach, for example) should not be used together with RX buffer (because this kind of reaction would produce cyanide)!

Add 60 ml (50 preps) or 180 ml (250 preps) 98-100% ethanol to WS Buffer.

Pipet a required volume of RX Buffer into another tube and add 10 µl β-mercaptoethanol (β-ME) per 1 ml RX Buffer before use.

| | |
|---|---|
| | **Sample Pre-Prep (case-dependent)** |
| Mix with: | **x µl RX** |
| Mix with: | **x µl EtOH** |
| Bind to Column: | **Load and Spin in RNA Column** |
| Wash with: | **500 µl WF** |
| Wash with: | **700 µl WS** |
| Remove residual EtOH: | **Centrifuge 3'** |
| Elute DNA in: | **30-50 µl H2O** |
| | **Down Stream Application** |



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# 2

## Overview

GenCatch™ Total RNA Extraction Miniprep System provides an economical method to purify total RNA from various samples such as cultured cells, tissues, and bacteria. A simple silica-membrane spin-column method can isolate total RNA without need of performing time-consuming phenol/ chloroform extraction and ethanol precipitation. Total RNA longer than 200 nucleotides are isolated, while small RNA such as 5.8S RNA, 5S RNA, and tRNA, which make up 15-20% of the total RNA, are excluded.

Binding Capacity and Typical Yield:
The binding capacity of the GenCatch™ total RNA mini column is 100 μg of total RNA, in order to avoid exceeding the binding capacity, use the sample preparation guide listed below:

| Sample | Recommended amount of sample | | Yield (μg) |
|---|---|---|---|
| Animal cells | NIH-3T3 | 1 x $10^6$ cells | 12 |
| | HeLa | 1 x $10^6$ cells | 15 |
| | COS-7 | 1 x $10^6$ cells | 30 |
| | LMH | 1 x $10^6$ cells | 12 |
| Animal tissues | Mouse/rat tissues | | |
| | Embryo | 10 mg | 30 |
| | Heart | 10 mg | 10 |
| | Brain | 10 mg | 10 |
| | Kidney | 10 mg | 35 |
| | Liver | 10 mg | 45 |
| | Spleen | 10 mg | 35 |
| | Lung | 10 mg | 10 |
| | Thymus | 10 mg | 45 |
| Bacteria | E. coli | 1 x 109 cells | 45 |
| | B. subtilis | 1 x 109 cells | 40 |

Downstream Applications:

- Northern-blotting
- RT-PCR
- PolyA+ RNA selection
- cDNA synthesis
- Primer Extension
- In vitro translation



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# 3

## Product Contents

GenCatch™ Total RNA Extraction Miniprep Kit contains sufficient reagents for 50 (Cat. No. 1660050) and 250 (Cat. No. 1660250) total RNA extraction applications, respectively.

| Catalog Number | 1660050<br>50 preps | 1660250<br>250 preps |
|---|---|---|
| RX Buffer | 36 ml | 150 ml |
| WF Buffer | 30 ml | 150 ml |
| WS Buffer | 15 ml | 45 ml |
| RNase-free ddH2O | 3 ml | 25 ml |
| GenCatch™ Column | 50 pieces | 250 pieces |
| Collection Tube | 50 pieces | 250 pieces |
| Elution Tube | 50 pieces | 250 pieces |

WARNING, strong acids and oxidants (bleach, for example) should not be used together with RX buffer (because this kind of reaction would produce cyanide)!

Add 60 ml (50 preps) or 180 ml (250 preps) 98-100% ethanol to Buffer WS before use (see instructions on bottle label).

Storage Conditions:
Store at room temperature

All components are guaranteed for 24 months from the date of purchase, when stored under specified conditions and used as described in this manual. Long term storage of some of the buffer may harden the HDPE plastic bottle. However this will not adversely affect the performance of the kit. GenCatch™ columns should be kept sealed in the zip lock bag provided during storage and away from any heating source.



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# 4

## Protocol

First time users are strongly recommended to read through this detailed protocol instruction.
For technical support please reach us at support@epochlifescience.com

Before you start:
Add 60 ml (50 preps) or 180 ml (250 preps) 98-100% ethanol to Buffer WS before use (see instructions on bottle label).

Please read the following notes before starting the procedures.

-Pipet a required volume of RX Buffer into another tube and add 10 μl β-mercaptoethanol (β-ME) per 1 ml RX Buffer before use.

-Complete disruption and homogenization of sample is essential for total RNA extraction.

-All plastic ware and containers should be treated properly to make sure RNase-free. Wear gloves when handling RNA.

-Buffers provided in this system contain irritants. Wear appropriate safety apparels such as gloves and lab coat.

-All centrifugation steps except cell pelleting are done at full speed (10,000 x g or 13,000-14,000 rpm) in a microcentrifuge.

-Some genomic DNA (and plasmid DNA, if any) will also be copurified with RNA. DNase treatment is therefore required when DNA-free RNA is desired. DNase can then be removed by phenol/chloroform extraction (refer to Protocol for "Removal of genomic DNA in eluted total RNA by DNase").

## I. Animal Tissue Protocol:

1. Add 350 μl RX Buffer (β-ME added) to 10 mg of liquid nitrogen-frozen or fresh tissue. Disrupt and homogenize the sample by grinding and shearing using 20-G needle syringe or Epoch Life Science's Shearing Tube.

2. Add 10 μl β-mercaptoethanol (β-ME) per 1 ml of Buffer RX. If using 20 mg of tissue add 700 μl of Buffer RX. If using Epoch Life Science's Shearing Tube, refer to "Application of Shearing Tube" section on the last page.



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3. Centrifuge the lysate for 5 minutes to spin down insoluble materials and use only the supernatant in the following steps.

4. Determine the final volume of the supernatant. Add an equal volume of 70% ethanol to the clear lysate and mix by vortexing.
   If lysate is lost during the preparation, reduce the volume of ethanol accordingly. Do not centrifuge the ethanol added lysate.

5. Place a Total RNA Mini Column onto a Collection Tube. Add 700 µl of the ethanol-added sample (including any precipitate) into the column. Centrifuge for 30-60 seconds. Discard the flow-through.
   Repeat this step for the rest of the sample. If some sample still retains in the column, repeat centrifugation until all sample pass the column.

6. Wash the column once with 0.5 ml WF Buffer by centrifuging for 30-60 seconds. Discard the flow through.

7. Wash the column once with 0.7 ml WS Buffer by centrifuging for 30-60 seconds. Discard the flow-through.
   Add 60 ml (50 preps) or 180 ml (250 preps) of 98-100% ethanol into WS Buffer bottle when first open.

8. Centrifuge the column for another 3 minutes to remove ethanol residue.

9. Place the column onto a 1.5-ml RNase-free Elution Tube. Add 30-50 µl RNase-free ddH2O (provided) onto the center of the membrane.
   For effective elution, make sure that the elution solution is dispensed onto the center of the membrane.

10. Stand the column for 1 minute, and centrifuge for 1-2 minutes to elute total RNA.

11. Store RNA at –70 °C.

## II. Animal Cells Protocol:



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1. Pellet 1 to 5 x 106 cells by centrifuging at 300 x g for 5 minutes. Remove all the supernatant.

2. Disrupt cells by adding 350 µl RX Buffer (β-ME added) to the cell pellet and vortex the sample. Homogenize the sample by using 20-G needle syringe or Epoch's Shearing Tube (not included, purchase separately Cat. No.: 2610-050).
   Add 10 µl β-mercaptoethanol (β-ME) per 1 ml of RX Buffer. If using Epoch's Shearing Tube, apply the disrupted lysate to a Shearing Tube and centrifuge for 1 minute to shear genomic DNA.

3. Follow the Animal Tissue Protocol from Step 2

## III. Animal Cell Cytoplasm Protocol:

1. Prepare cytoplasm lysate
   Prepare cell lysis buffer (provide by user):

   20 mM Tris-HCl pH 8.0, 1 mM MgCl2, 0.5% NP-40.Keep at 4°C.

   Only fresh cells are used for preparing cytoplasm lysate.

   a. Harvest 5 x $10^6$ - 1 x $10^7$ cells and centrifuge at 300x g to pellet cells.

   b. Add 180 µl of cell lysis buffer to the cell pellet, resuspend and lysis cells by gentle pipetting. Incubate the lysate on ice for 5 minutes.

   c. Centrifuge the lysate at 300 x g at 4°C for 3 minutes, transfer the supernatant to a new tube, and use the supernatant (lysate) in the following steps.

2. Add 600 µl of RX Buffer (β-ME added) to the lysate and mix by vortexing.
   Add 10 µl β-mercaptoethanol (β-ME) per 1 ml of RX Buffer.

3. Add 450 µl of 98% ethanol to the sample and mix by vortexing.

4. Follow the Animal Tissue Protocol starting from Step 4.



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## IV. Bacteria Protocol:

1. Pellet up to 1 x $10^9$ bacterial cells by centrifuging at 5,000 x g (7,500 rpm) for 5 minutes. Remove all the supernatant.

2. Resuspend cells in 100 µl of TE buffer by vortexing.

3. Add lysozyme (provide by user) to a final concentration of 500 µg/ml for Gram-negative bacteria; 2 mg/ml for Gram-positive bacteria, and incubate at room temperature for 10 minutes.

4. Add 350 µl RX Buffer (β-ME added) to the sample and mix by vortexing.
   Add 10 µl β-mercaptoethanol (β-ME) per 1 ml of Buffer RX.

5. Centrifuge lysate for 5 minutes to spin down the insoluble materials and use only the supernatant in the following steps.

6. Add 250 µl of 98 % ethanol to the sample and mix by vortexing.

7. Follow the Animal Tissue Protocol starting from Step 4.

## V. Removal of genomic DNA in eluted total RNA by DNase:

1. Incubate total RNA with RNase-free DNase I (1 unit per µg of total RNA) in 50 mM Tris-HCl (pH 7.5), 10 mM MgCl2, and 50 µg/ml BSA at 37 °C for 15-30 minutes.

2. Remove DNase I by adding an equal volume of phenol: chloroform (1:1) and mix well. Centrifuge for 5 minutes. Transfer the upper aqueous layer to a new eppendorf tube.

3. Add 1/10 volume of 3 M sodium acetate (pH 5.2) and 1 volume of ice-cold isopropanol to the solution and mix well. Chill on ice for 30 minutes.

4. Centrifuge for 10 minutes at 4°C. Discard the supernatant. Wash the pellet twice with 1 ml of 70 % ethanol and recentrifuge.

5. Remove all supernatant. Air dry the RNA pellet. Redissolve RNA in RNase-free dd$H_2O$.



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## VI. The of Shearing Tubes:

Shearing Tube (not included, purchase separately, Cat. No.: 2160-050) is designed for simple and fast homogenization of tissue and cell lysate. The lysate is loaded into a Shearing Tube sitting in a 2-ml Collection Tube and centrifuge the tube for 1-2 minutes at full speed (10,000 x g or 13,000-14,000 rpm) in a microcentrifuge. When collecting homogenized lysate from the Collection Tube, avoid pipetting any debris and pellet formed at the bottom of the tube.



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# 5 Trouble Shooting

The following guide addresses some of the most common problems. For more specific questions please reach us at support@epochlifescience.com

### Little or no RNA eluted:

a. Insufficient disruption or homogenization:
Reduce the amount of starting sample and perform more disruption and homogenization.

b. Clogged column:
Reduce the amount of starting sample and perform more disruption and homogenization. Centrifuge the lysate to remove insoluble materials and use the supernatant only.

c. RNA is degraded:
Starting sample should be fresh or frozen in liquid nitrogen and store at –80 ℃. Improper handling of the sample or storing the sample at –20℃ will cause RNA degradation.

d. RNase contamination:
Use RNase-free solution, pipette tips and centrifuge tubes.

### DNA contamination:

Refer to Protocol section V: "Removal of genomic DNA in eluted total RNA by DNase"

### A260/A280 ratio of eluted RNA is low:

a. Use dd$H_2O$ of acidic pH to dilute RNA sample for spectrophotometric analysis:
Use 10 mM Tris-HCl of pH 7.5 or TE buffer to dilute RNA sample.

b. DNA is copurified with RNA:
Refer to protocol section V: "Removal of genomic DNA in eluted total RNA by DNase"



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