1760250-2/23

GenCatch™ Plant Total RNA Miniprep Kit

User's Guide for

Total RNA Extraction From Plants and Fungi

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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.

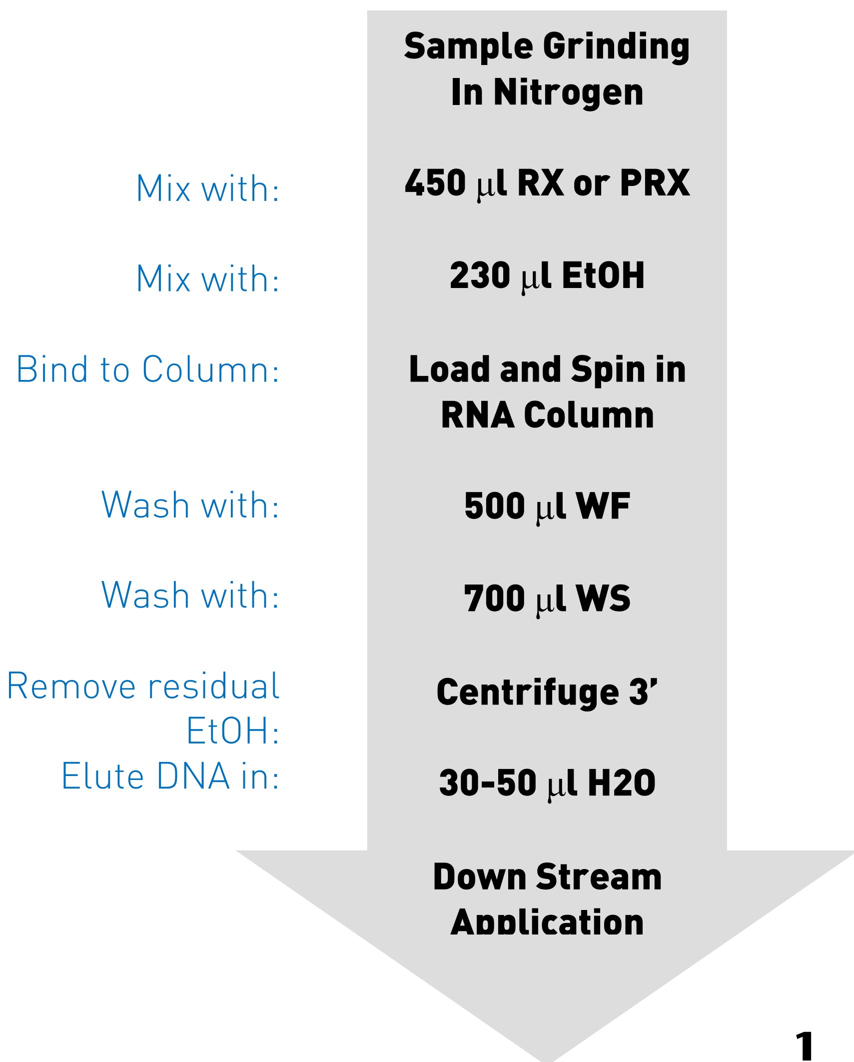

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Table of Contents

Quick Start Procedure	1
Overview	3
Product Content	4
Protocol	5
Troubleshooting Guide	9

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2

Overview

GenCatchTM Plant Total RNA Miniprep System provides an economical method to purify total RNA from various plant samples such as cultured plant cells, plant tissues, leafs and seeds etc. 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™ Plant Total RNA mini column is 100 µg of total RNA, in order to avoid exceeding the binding capacity, please use 100 mg plant material or 1 x 10^7 cells only.

Downstream Applications:

Plant RNA prepared using GenCatchTM Plant Total RNA Miniprep Kit is suitable for a variety of molecular manipulations:

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

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3

Product Contents

GenCatchTM Plant Total RNA Miniprep Kit contains sufficient reagents for 50 (Cat. No. 1760050) and 250 (Cat. No. 176250) plant total RNA extraction applications, respectively.

Catalog Number	1760050	1760250
Size (preps)	50	250
RX Buffer	27ml	135ml
PRX Buffer	27ml	135ml
WF Buffer (RNA)	30ml	150ml
WS Buffer (RNA)	15ml	45ml x2
RNase-free ddH2O	1.5ml x2	15ml
RNA Mini Column	50	250
Collection tube	100	500
RNA Shearing Tube	50	250
Elution Tube	50	250

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.

If precipitate is observed for any of the buffer, warm the bottles to 37°C until it is dissolved.

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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 (for Cat. No. 1760050) or 180ml (for Cat. No. 1760250) of ethanol (98-100%) to the WS Buffer bottle when first open the bottle.

Add 10 µl β-mercaptoethanol (β-ME) per 1 ml RX Buffer or PRX Buffer.

DO NOT MIX RX BUFFER WITH STRONG ACIDS OR OXIDANTS SUCH AS BLEACH. (THIS WILL LEAD TO THE PRODUCTION OF CYANIDE)

The binding capacity of the GenCatch™ Plant Total RNA mini spin column is 100 µg of total RNA. In order to avoid exceeding the binding capacity, use 100 mg plant material or 1 x 10^7 cells only.

I. Protocol:

1. Grind 100 mg (or less) plant sample under liquid nitrogen to a fine powder and transfer to a new tube.

2. Add 450 µl of RX Buffer or PRX Buffer (β-ME added) to the tissue powder and vortex vigorously.
   In most cases RX Buffer is the buffer of choice to lyse plant tissue. However, plant tissues contain sticky secondary metabolites (for example, maize with milky endosperm or mycelia of filamentous fungi), PRX Buffer is used instead.

3. Apply lysate to the Shearing Tube sitting in a Collection Tube and centrifuge at full speed (13,000 rpm or 10,000 x g) for 2 - 10 minutes. Transfer flow-through sample from the Collection Tube to a new tube.
   Avoid pipetting any debris and pellet in the collection tube.

4. Add 230 µl (about half of the sample volume) 98-100% ethanol to the clear lysate and mix by pipetting.
   If sample lysate is lost during the preparation, reduce ethanol volume proportionally.

5. Apply 680 µl of the ethanol added sample (including any

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precipitate) from step 4 to a Plant Total RNA Mini Column sitting in a Collection Tube, close the cap, centrifuge at 8,000 x g (10,000 rpm) for 1 minute, and discard the filtrate.
If the solution remains above the membrane, centrifuge again at 13,000 rpm.

6. Repeat step 5 for rest of the sample.

7. Wash the column once with 0.5 ml of WF Buffer by centrifuging at full speed for 30-60 seconds and discard the filtrate.

8. Wash the column twice with 0.7 ml of WS Buffer by centrifuging at full speed for 30-60 seconds and discard the filtrate.
Add 60 ml (for 1760050) or 180 ml (for 1760250) of ethanol (98-100%) to each WS Buffer bottle when first opened.

9. Centrifuge at full speed for 3 minutes to remove traces of WS Buffer.
Residual ethanol may inhibit reverse transcriptase activity.

10. Transfer the column to a RNase-free 1.5 ml Elution Tube, add 50 µl of RNase-free ddH2O, and centrifuge at full speed for 1 - 2 minutes to elute RNA.

11. Store RNA at -70°C.

II. 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.

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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 ddH_2O.

III. The of Shearing Tubes:

Shearing Tube 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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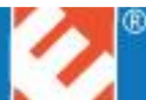

5

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 II: "Removal of genomic DNA in eluted total RNA by DNase"

A260/A280 ratio of eluted RNA is low:

a. Use ddH_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 II: "Removal of genomic DNA in eluted total RNA by DNase"

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