



# **Total RNA Extraction Kit (For Tissue and Cell)**

## **User Manual**

**Catalog # CRG1021**

Developed and optimized for total RNA isolation in fresh tissue samples or cell samples.

**For research use only. Not for diagnostic or therapeutic procedures.**

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## I. INTRODUCTION

Total RNA Extraction Kit (For Tissue and Cell) integrates traditional phenol/guanidine-based lysis and silica-gel-membrane purification of total RNA. The kit also provides lysis buffer, designed to facilitate lysis of samples and inhibit RNase. After RNA binding to the silica membrane, phenol and other contaminations are efficiently washed away by the wash buffer in the kit. Then high quality of total RNA is eluted in 50 µl or more RNase-free water.

## II. KIT COMPONENTS

Component	20 Preps	50 Preps	Storage
Mini-spin Column	20	50	RT
1.5 ml RNase-free Tube	20	50	RT
2.0 ml Collection Tube	20	50	RT
Lysis Buffer	24 ml x 1	30 ml x 2	RT
Wash Buffer (5X)	5.5 ml x 2	5.5 ml x 5	RT
RNase-free Eution Buffer	2 ml x 1	5 ml x 1	RT
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### Note:

Wash Buffer (5X): For each bottle, add 22 ml Ethonal to dissolve before use.

## III. STORAGE AND STABILITY

All kit components are stable at room temperature for 12 months.

#### **IV. MATERIALS REQUIRED BUT NOT PROVIDED**

1. Sterile, RNase-free tips
2. Disposable gloves
3. Equipment and tubes for tissue disruption and homogenization
4. RNase-free 1.5 ml or 2 ml microcentrifuge tubes
5. Microcentrifuges for centrifugation at 4 °C and room temperature
6. Chloroform
7. 95% ~ 100% Ethonal
8. RNase-free water/DEPC water

## V. PROCEDURE

**1.** To extract RNA from tissues, cut a piece of deep-frozen tissue in a chilled mortar, and weight by electronic balance. Transfer tissue immediately into a 1.5 ml RNase-free tube which already contains 1 ml of Lysis Buffer. Homogenize tissue with an electronic homogenizer on ice for 15 s or until no visible tissue, and then move to step 3.

**Note:** Tissue sample should be stored in liquid nitrogen or in deep freezer (-80 °C).

This protocol is suitable for isolation of RNA from less than 50 mg of tissue; otherwise, more Lysis Buffer is needed to digest tissue sample.

**2.** To extract RNA from cells, put cell culture dish or flask on ice, remove culture medium, and wash cells twice with ice-cold 1X PBS. Add 1 ml of Lysis Buffer and scrape cells off the plate with cell lifter. Transfer cell lysates into 1.5 ml RNase-free tube, vortex thoroughly for 30 s, and then move to step 3.

**Note:** PBS buffer is not provided with kit, and is available separately.

For suspension cells, spin down cells first at 2,000 x g for 3 min, wash cells twice with 1X PBS, and then add 1 ml of Lysis Buffer.

This protocol is suitable for isolation of RNA from  $< 1 \times 10^7$  cells, or from a  $< 10$  cm diameter dish; otherwise, more Lysis Buffer is needed to homogenize cells.

**3.** Incubate mixture at room temperature for 5 min, and add 0.2 ml of chloroform. Cap the tube securely, and shake it vigorously for 15 s.

**Note:** Avoid vortexing as this may increase the DNA contamination to the RNA sample.

**4.** Place the tube at room temperature for another 2 - 3 min, and centrifuge at 12,000 x g for 15 min at 4 °C.

**Note:** Centrifugation separates solution into three phases: upper aqueous with RNA, middle layer with protein/DNA, bottom organic with DNA. The 4 °C is essential for phase separation.

5. Transfer the upper aqueous phase to a new 1.5 ml sterile tube, and add 1 volume of 70 % ethanol. Mix solution thoroughly by vortex for 5 s.

**Note:** Discard the middle layer and bottom organic phase if no need for isolation of DNA.

6. Pipet up to 700  $\mu$ l of the sample immediately, including any precipitate that may have formed, into a mini-spin column in a 2 ml collection tube. Close tube gently, and centrifuge at 8,000 x g for 15 s at room temperature. Discard the flow-through.

7. Repeat step 6 using the remaining sample, and discard the flow-through.

8. Add 700  $\mu$ l of Wash Buffer into a mini-spin column. Close the tube gently, and centrifuge at 8,000 x g for 15 s. Discard the flow-through.

**Note:** Ensure that three volume of 100 % ethanol is added to Wash Buffer before using for the first time.

9. Repeat step 8 twice with 500  $\mu$ l Wash Buffer. After last wash, centrifuge column for 2 min at 8,000 x g to dry silica-gel membrane.

**Note:** It is important to completely remove washing solution. Any carryover may affect elution.

10. Transfer column into a new RNase/DNase-free 1.5 ml tube, pipet 40  $\mu$ l RNase/DNase-free water directly onto the RNeasy silica-gel membrane. Close the tube gently, wait 1 to 5 min, and centrifuge for 1 min at 8,000 x g to elute RNA.

11. Load elute back to the column, and repeat the elution step 10.

12. Keep eluted RNA sample on ice all the time and store at -70 °C.

## VI. TROUBLESHOOTING GUIDE

PROBLEM	GUIDE
<b>Insufficient homogenate and mix</b>	This is usually caused by insufficient mix after addition of chloroform, or by centrifugation at a high temperature. As described in step 3 and 4 of the protocol, you need to shake the tube vigorously for at least 15 s after addition of chloroform. Centrifuge the tube at 4 °C or no higher than 80C. A higher temperature will disrupt phase separation.
<b>Insufficient homogenate</b>	Insufficient homogenate may result in large tissue piece that may clog the column. Please increase the g-force and time of centrifugation if necessary.
<b>Too much starting material</b>	The starting material can not be more than 100mg tissue. Please reduce the amount of starting material if necessary.
<b>Sample or material stored in bad condition</b>	Total RNA in cells or tissues that in bad condition or dealt with some medication may lead to self-degradation. Please store samples in -80 °C or liquid nitrogen. It should be better if some commercial RNase inhibitor such as RNA Later.
<b>Sample Collection</b>	It is important to inactivate RNase as soon as possible once samples are gathered. Please shorten the time of collection as possible as you can to yield higher quality of total RNA.
<b>Sample manipulation before lysis</b>	Keep tissue frozen before lysis. Please transfer tissue sample into lysis buffer contains RNase inhibitor before thawing and homogenize it in ice bath immediately.
<b>Centrifugation</b>	Please centrifuge samples at 4 °C after adding chloroform.
<b>Degradation after electrophoresis</b>	If loading RNA is more than 5 µg, the band will show some smear. So please take about 700 ng to 800 ng total RNA for electrophoresis.
<b>Exogenous RNase contamination</b>	Disposable gloves and respirator must be used during the whole process to prevent any RNase contamination. The mortar and muller should be dried and heated at 180 °C over night before use to eliminate RNase. All the tubes and tips needed should be treated by DEPC water and then autoclave them before use.
<b>Low Yield</b>	It is normal to see the variation of RNA yield. However, an extreme low yield of RNA may indicate failure of extraction. Many factors can reduce the yield of RNA, such as poor quality of tissue samples, insufficient homogenization, and poor elution. It is important to select good quality tissue and handle it appropriately. Complete homogenization in Ezol reagent is also essential for extraction of RNA. In some cases, a low yield of RNA is caused by poor elution, and an extended incubation with RNase-free water on column may enhance RNA recovery.
<b>Low A260/A280 Value</b>	Usually, a low A260/A280 value is caused by measurement of absorbance in water, or by contamination of proteins, phenol, or other organic chemicals. Under normal conditions, the isolated RNA should be free of phenol and organic chemicals. However, insufficient homogenization may cause co-purification of proteins and increase reading at 280 nm. We recommend following the described procedures to digest and homogenize tissue samples. We also recommend measuring the ratio of A260/A280 in 10 mM Tris-HCl buffer.

## **VII. TECHNICAL SUPPORT**

For troubleshooting, information or assistance, please go online to [www.cohesionbio.com](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## **VIII. NOTES**