Total RNA Extraction Kit

(For Serum and Plasma)

User Manual

Catalog # CRG1022

Developed and optimized for total RNA isolation in serum or plasma samples.

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

I. INTRODUCTION

Total RNA Extraction Kit (For Serum and Plasma) provides lysis buffer, designed to facilitate lysis of samples and inhibit RNase. After RNA released, 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
1.5 ml RNase-free 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
Carrier RNA	150 µl x 1	360 µl x 1	-20 °C
Glycogen	25 µl x 1	60 µl x 1	-20 °C
cel-mir-39-3p	1.2 ml x 1	1.5 ml x 2	-20 °C
RNase-free Eution Buffer	2 ml x 1	5 ml x 1	RT
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Note:

cel-mir-39-3p: Act as a external reference gene to control the degradation of RNA in serum/plasma and the quality of RNA extraction process.

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

III. STORAGE AND STABILITY

Carrier RNA, Glycogen and cel-mir-39-3p should be stored at -20 °C.

Other 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. Eqiupment 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. Isopropanol
8. RNase-free water/DEPC water

V. PROCEDURE

1. The level of RNA in serum or plasma is very low, so transferred and stored serum or plasma in a RNase-free tube is strongly recommended.

2. To extract RNA, put serum or plasma sample on ice. Add 800 µl of Lysis Buffer and 50 µl cel-mir-39-3p solution and 6 µl Carrier RNA in 1.5 ml RNase-free tube. Transfer 200 µl serum or plasma sample into Lysis Buffer, vertex thoroughly for 30 s, and then move to step 3.

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 vertexing 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 20 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 isopropanol and 1 µl Glycogen. Mix solution thoroughly by vortex for 5s. Place the tube at -80 °C overnight.

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

Place the tube at -80 °C overnight is very important to precipitate RNA in the serum and plasma.

6. Take out the tube from -80 °C and centrifuge at 12,000 x g for 20 min at 4 °C, discard the supernatant

7. Add 1mL Wash Buffer (1X) to precipitation and resuspended for several times. Centrifuge at 12,000 x g for 5 min at 4 °C and discard the supernatant.

8. Repeat step 7 once and dry the precipitation.

9. Pipet 50 µl RNase-free Eution Buffer r to the precipitation wait 1 to 5 min to elute RNA.

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

VI. TROUBLESHOOTING GUIDE

PROBLEM	GUIDE
Insufficient homogenate and mix	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 8oC. A higher temperature will disrupt phase separation.
Insufficient homogenate	Insufficient homogenate may result in large tissue piece that may clog the column. Please increase the g-force and time of centrifugation if necessary.
Too much starting material	The starting material can not be more than 100mg tissue. Please reduce the amount of starting material if necessary.
Sample or material stored in bad condition	Total RNA in cells or tissues that in bad condition or dealed 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.
Sample Collection	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.
Sample manipulation before lysis	Keep tissue frozen before lysis. Please transfer tissue sample into lysis buffer contains RNase inhibitor before thawing and homogenize it in ice bath immediately.
Centrifugation	Please centrifuge samples at 4 °C after adding chloroform.
Degradation after electrophoresis	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.
Exogenous RNase contamination	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.
Low Yield	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.
Low A260/A280 Value	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 or contact us at techsupport@cohesionbio.com

VIII. NOTES