Version: 10/2023

RNApure Circulating Reagent

Cat. No. : CW2281S (50 preps)
Storage Conditions: 2-8°C, avoid light

Components

Component	CW2281S 50 preps
RNApure Circulating Reagent	50 mL

Introduction

Cell-free RNA (serum, plasma and urine) extraction reagents are particularly suitable for the isolation and purification of total RNA, including microRNA and small RNA (< 200 nt), from serum and plasma. This product is flexible in handling samples of different starting quantities, it and can effectively preserve the integrity of RNA when effectively lysing the sample. The extracted total RNA has good integrity, no protein and DNA contamination, and the extracted RNA can be used in downstream experiments such as RT-PCR, Northern Blot and molecular cloning.

Reagents to Be Supplied by User

chloroform, isopropyl alcohol, 75% ethanol, RNase-free water (newly opened or special for RNA extraction).

Precautions

1. Prevention of RNase pollution, should pay attention to the following aspects:

1.1 Use RNase-free plastic products and tips to avoid contamination.

1.2 Glassware should be sterilized at 180°C for 4 hours before use, plastic ware can be soaked in 0.5 M NaOH for 10 min, rinse thoroughly with water and autoclave.

1.3 The solution should be prepared using RNase-free water.

1.4 Operators should wear disposable masks and gloves, and change gloves routinely during the experiment.

2. The extracted samples should avoid repeated freeze-thawing, which would affect the yield and quality of RNA extraction.
3. This product contains phenol, which is toxic and corrosive. If inhaled into the body, in contact with skin, or swallowed, it may cause poisoning, burns, and other physical harm. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. In case of accidental contact with the eyes, immediately flush with plenty of water and go to the hospital for treatment.
4. Samples are homogenized with cell-free RNA (serum, plasma and urine) extraction reagent and left at -70°C for more than one month if chloroform is not added immediately.
5. RNA precipitates stored in 75% ethanol can be preserved for one week at 2-8°C, and for one year at -20°C. RNA has a short half-life and is easy to degrade. It is suggested to conduct follow-up experiments as soon as possible after extraction, such as reverse transcription into cDNA and Northern Blot.
6. If the downstream assay is very sensitive to DNA, it is recommended that the RNA be treated with DNase I (Cat. No.: CW2090) without RNase.

Protocol

1. 200 μL fresh or frozen serum or plasma is taken, and three times volume of cell-free RNA (serum, plasma and urine) extraction reagent is added. Shake for 30 s and mix well.
   Note: After adding cell-free RNA (serum, plasma and urine) extraction reagent to the sample, precipitation may occur. After shaking and mixing, precipitation will basically disappear. If there is still a small amount of precipitation, this does not affect the downstream experiment and can continue to operate.
2. The treated sample was left at room temperature for 5 min to completely separate the protein and nucleic acid complexes.
3. Chloroform was added to the above solution, 0.2 mL chloroform was added for every 1 mL total RNA extraction reagent for serum/plasma samples, the tube was covered, violently shook for 15 s, and left at room temperature for 2-3 min.
   Note: If the solution cannot be mixed by vortexing, it can be mixed manually by rapid inversion for 2 min.
4. After centrifugation at 4°C and 12,000 rpm for 20 min, the sample was divided into three layers: red organic phase, middle layer and upper colorless water phase. RNA was mainly in the water phase, which was transferred to a new RNase-free centrifuge tube (self-provided).
5. Add equal volume isopropyl alcohol to the aqueous solution obtained, mix upside down, and leave at room temperature for 30 min, or leave it at -20°C overnight for better results.
6. Centrifuge at 12,000 rpm at 4°C for 20 min and discard the supernatant.
   Note: RNA precipitates are often invisible before centrifugation and form gel precipitates on the side and bottom of the tube after centrifugation.
7. The precipitation was washed with 75% ethanol (prepared in RNase-free water). Every 1 mL of cell-free RNA (serum, plasma and urine) extraction reagent was added to 1 mL of 75% ethanol to wash the precipitation.
8. Centrifuge at 4°C at 12,000 rpm for 3 min. Carefully discard the supernatant and do not discard the RNA precipitation.
   Note:The remaining small amount of liquid can be centrifuged for a short time, and then sucked out with the gun head. Be careful not to discard the precipitation.
9. Let stand at room temperature for 2-3 min to dry. The RNA was fully dissolved by adding 30-100 μL RNase-free water, and the resulting RNA was stored at -70°C to prevent degradation.
   Note:The precipitation should not be too dry, so as not to dissolve.