# Free RNA (serum plasma urine) extraction reagent

Project number: R665870

**Storage conditions:** 2-8° C.

**Product Content:**

| Component | R6658705 0preps |
|---|---|
| RNApureCirculatingReagent | 50ml |

## Product Description:

Free RNA (serum plasma urine) Extraction Reagent is especially suitable for the isolation and purification of total RNA including microRNA and other small RNA (<200nt) from serum and plasma. The product is flexible to handle samples with different starting amounts, and can effectively preserve the integrity of the RNA while lysing the sample. The extracted total RNA has good integrity and is free of protein and DNA contamination. The product can be used in downstream experiments such as RT-PCR, NorthernBlot and molecular cloning. The extracted RNA can be used in downstream experiments such as RT-PCR, NorthernBlot and molecular cloning.

**Self-contained reagents:** chloroform, isopropanol, 75% ethanol, RNase-free water (freshly opened or for RNA extraction).

## Caveats:

1. To prevent RNase contamination, attention should be paid to the following aspects:

1) Use RNase-free plastics and tips to avoid cross-contamination.

(2) Glassware should be dry baked at 180℃ for 4 hours before use, and plasticware can be soaked in 0.5MNaOH for 10 hours.

minutes, rinse thoroughly with water and autoclave.

3) RNase-free water should be used to prepare the solution.

(4) Operators wear disposable masks and gloves, and change gloves diligently during the experiment.

2. Avoid repeated freezing and thawing of the extracted samples, otherwise it will affect the rate and quality of RNA extraction.

3. This product contains phenol, which is toxic and corrosive. It can cause poisoning, burns and other bodily injury if inhaled, in contact with the skin, or swallowed. Protective items, such as protective clothing, gloves, eye protection, face masks, etc., should be worn when using this product. In case of accidental contact with eyes, flush immediately with plenty of water and go to hospital for treatment.

4. After the sample is homogenized with the free RNA (serum plasma urine liquid)

extraction reagent, it can be left at -70° C for more than one month if chloroform is not added immediately.

5. RNA precipitation in 75% ethanol can be stored at 2-8° C for one week and at -20° C for one year.RNA has a relatively short half-life and is easily degraded, so it is recommended to carry out subsequent experiments as soon as possible after the extraction, e.g., reverse transcription into cDNA, NorthernBlot, etc.

6. If downstream experiments are very sensitive to DNA, it is recommended that RNA be treated with RNase-free DNaseI.

**procedure**

1. Take 200 μl of fresh or frozen serum or plasma and add 3 times the volume of free RNA (serum plasma urine) extraction reagent. Shake for 30 seconds and mix well.

Note: After the sample is added to the free RNA (serum plasma urine) extraction reagent, precipitation may appear, which basically disappears after shaking and mixing. If there is still a small amount of precipitation, it does not affect the downstream experiments and the operation can be continued.

2. The treated samples were left at room temperature for 5 minutes to allow complete separation of the protein-nucleic acid complexes.

3. Add chloroform to the above solution, add 0.2 ml of chloroform for every 1 ml of total RNA extraction reagent for serum/plasma samples, cover the tube with a cap, shake vigorously for 15 seconds, and leave at room temperature for 2-3 minutes.

Note: If vortex mixing is not possible, mix manually by quickly inverting for 2 minutes.

4. Centrifuge at 12,000 rpm for 20 minutes at 4° C. At this point, the sample is divided into three layers: a red organic phase, an intermediate layer, and an upper colorless aqueous phase, with the RNA predominantly in the aqueous phase, which is transferred to a new RNase-free centrifuge tube (self-provided).

5. Add an equal volume of isopropanol to the resulting aqueous solution, mix upside down and leave at room temperature for 30 minutes. Or precipitate at -20° C overnight for better results.

6. Centrifuge at 12,000 rpm for 20 minutes at 4° C and discard the supernatant.

Note: The RNA precipitate is often invisible before centrifugation and forms a gelatinous precipitate on the side and bottom of the tube after centrifugation.

7. Wash the precipitate by adding 75% ethanol (prepared with RNase-free water). The precipitate was washed by adding 1 ml of 75% ethanol for every 1 ml of free RNA (serum plasma urine) extraction reagent used.

8. Centrifuge at 12,000 rpm for 3 minutes at 4° C and carefully aspirate the

supernatant, taking care not to aspirate the RNA precipitate.

Note: The small amount of liquid remaining can be centrifuged briefly and then aspirated with a lance tip, taking care not to discard the precipitate.

9. Leave at room temperature for 2-3 minutes and dry. Add 30-100 μl of RNase-free water to fully dissolve the RNA, and store the obtained RNA at -70°C to prevent degradation.

Note: Do not over dry the precipitate as it may be difficult to dissolve.