Total RNA Isolation Reagent Kit

Catalogue No.:abx460023

Total RNA Isolation Reagent Kit provides an efficient 3-step method to isolate the total RNA from the tissue, cultured animal and bacterial cells, blood, and serum. This unique reagent system ensures the total RNA with a high yield and good quality from samples of unlimited size. If a larger sample is required, the reagent volume can be scaled proportionately, making this reagent not only very user-friendly but also highly versatile. RNA phenol extraction is not required, and the entire procedure can be completed in 60 minutes. The total RNA is ready for use in RT-PCR, Northern Blotting, cDNA Synthesis and Mapping.

Contents:

- Buffer 1: 50 ml
- Buffer 2: 6 ml

Reagents Required But Not Provided:

- Microcentrifuge and microcentrifuge tubes
- Water bath or incubator
- Mortar and pestle
- RNase A (50 mg/ml)
- RNase-free water
- Beta-mercaptoethanol
- Isopropanol
- Chloroform
- 70% EtOH (prepared from Absolute EtOH diluted in RNase-free water)

Target:	Total RNA Isolation Reagent Kit
Tested Applications:	RT-PCR
Storage:	Store at room temperature.
Buffer:	Not applicable.
Note:	THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Abbexa LTD, Cambridge, UK · Phone: +44 (0) 1223 755950 · Fax: +44 (0) 1223 755951
Abbexa LLC, Houston, TX USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
Website: www.abbexa.com · Email: info@abbexa.com

Directions for use:

Sample Preparation:

• **Tissue:** Grind 50 mg of fresh tissue in liquid nitrogen using a mortar and pestle.

• **Cultured Animal or Bacterial Cells:** Add 5 × 10^6 animal cells or 1 × 10^9 bacterial cells to a microcentrifuge tube. Centrifuge at 14,000 - 16,000 × g for 1 minute. Discard the majority of the supernatant (if more than 1.5 ml of bacterial cululture is used, repeat this step). Use the remaining supernatant to resuspend the pellet.

• **Fresh or Frozen Blood:** Collect blood in anticoagulant-treated collection tubes. Transfer the blood to a sterile tube (up to 300 µl to a 1.5 ml microcentrifuge tube; up to 1 ml to a 15 ml centrifuge tube).

Assay Procedure:

Before carrying out the assay, check the Buffers for any salt precipitation. Re-dissolve any precipitates by warming to 37 °C.

1. Lysis.

- **Tissue:** Add 500 µl of Buffer 1 and 8 µl of beta-mercaptoethanol to the sample. Grind the sample in the motar until it is completely dissolved. Transfer the dissolved sample to a microcentrifuge tube.
- **Cultured Animal or Bacterial Cells; Fresh or Frozen Blood:** Add 500 µl of Buffer 1 and 8 µl of beta-mercaptoethanol to the sample and mix fully.
- **Serum:** Add 500 µl of serum to a microcentrifuge tube. Add 500 µl of Buffer 1 and 8 µl of beta-mercaptoethanol to the sample and mix fully.

For frozen samples, incubate at 90 °C for 30 minutes, and then at 15-30 °C for 5 minutes. Centrifuge at 14,000 - 16,000 × g at 2-8 °C for 15 minutes, then transfer the supernatant to a new microcentrifuge tube. For all other samples, incubate at 60 °C for 10 minutes.

2. Phase Separation. To the supernatant obtained in the previous step, add Buffer 2 at 1/10 of the supernatant volume, followed by 500 µl of chloroform. Shake vigorously and then centrifuge at 14,000 - 16,000 × g at 2-8 °C for 10 minutes. Carefully remove the upper phase and transfer it to a new microcentrifuge tube. Repeat until the interphase becomes clear, then transfer the clear upper phase to a new microcentrifuge tube.

3. RNA Precipitation. To the clear upperphase obtained in the previous step, add 500 µl of isopropanol. Mix the sample by inverting gently. Allow to stand on ice for 10 minutes. Centrifuge at 14,000 - 16,000 × g at 2-8 °C for 15 minutes. Discard the supernatant and wash the pellet with 1 ml of 70% EtOH. Centrifuge at 14,000 - 16,000 × g at 2-8 °C for 5 minutes. Completely discard the supernatant and re-suspend the pellets in 50-100 µl of RNase-free water. Incubate for 10 minutes at 60 °C to dissolve the pellet.

Abbexa LTD, Cambridge, UK · Phone: +44 (0) 1223 755950 · Fax: +44 (0) 1223 755951
Abbexa LLC, Houston, TX USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
Website: www.abbexa.com · Email: info@abbexa.com