

EcoPURE Total RNA Micro Kit

50 rxns

Cat No: E2416

Shipping : Ship at ambient temperature.

Storage : Store the kit between 15°C and 25°C.

General Information

EcoPURE Total RNA Micro Kit is designed as a simple and convenient purification of high quality total RNA including small RNAs (e.g. microRNAs) from cultured cells and frozen or fresh tissues. This kit utilizes chaotropic ions and silica-based membrane technology, eliminating the need for expensive resins, hazardous phenol-chloroform extractions, β -mercaptoethanol, or time-consuming alcohol precipitation. The standard protocol lasts less than 10 minutes at room temperature and purified RNA can be effectively used in routine downstream applications including cDNA synthesis, northern blotting, differential display, primer extension, and mRNA selection.

Kit Contents

<i>EcoPURE</i> Micro Lysis/Binding Buffer	(22 ml)
<i>EcoPURE</i> Micro Wash Buffer 1	(22 ml)
<i>EcoPURE</i> Micro Wash Buffer 2*	(10 ml concentrate)
<i>EcoPURE</i> Micro Elution Buffer	(5 ml)
<i>EcoPURE</i> Micro Spin Columns	(50)
<i>EcoPURE</i> Collection Tubes	(50)

*Add 40 ml absolute ethanol

Protocol for Suspension or Adherent Cell Total RNA

Each isolation procedure is suitable for isolation of total RNA from 10^3 to 2.5×10^5 cells.

1a. Suspension Cell Cultures: Pellet appropriate number of suspension cells for 5 minutes at 1200 rpm.

1b. Adherent Cell Cultures: Trypsinize and pellet appropriate number of adherent cells for 5 minutes at 1200 rpm before lysis.

2. Aspirate media and wash cell pellet with an appropriate amount of 1x PBS.

3. Carefully decant the supernatant.

Note: For inputs of fewer than 10^5 cells, 30-50 μ l of media may be left behind in order to ensure that the pellet, which could be invisible, is not dislodged.

Note: Cell pellets can be stored at -80°C for later use. Frozen pellets should be stored maximally for 2 weeks to ensure that the integrity of the RNA is not compromised. Frozen cell pellets should not be thawed prior to beginning of the protocol. Frozen cell pellets should be immediately lysed with *EcoPURE* Micro Lysis/Binding Buffer.

4. Add 400 μ l *EcoPURE* Micro Lysis/Binding Buffer to pelleted cells. Mix well by pipetting. Then, vortex for 10 seconds until the entire pellet is completely dissolved before proceeding to the next step.

Precaution: *EcoPURE* Micro Lysis/Binding Buffer has the ability to inactivate the RNases in cells, however, 4 μ l β -mercaptoethanol might be added to each 400 μ l *EcoPURE* Micro Lysis/Binding Buffer for cells known to have high RNase activity to ensure the inactivation of RNases.

5. Add 400 μ l absolute (96-100%) ethanol to the lysate and mix well by vortexing for 10 seconds.

6. Insert an *EcoPURE* Micro Spin Column into a Collection Tube and transfer 700 μ l sample from step 5 to the *EcoPURE* Micro Spin Columns. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature. Depending on your lysate volume, repeat Step 6 as necessary.

Optional: *EcoPURE* Total RNA Micro Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional On-Column DNA Removal might be applied for maximum removal of residual DNA that may affect sensitive downstream applications.

7. Discard the flow through and add 400 μ l *EcoPURE* Micro Wash Buffer 1 to the *EcoPURE* Micro Spin Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

8. Discard the flow through and add 500 μ l *EcoPURE* Micro Wash Buffer 2 to the *EcoPURE* Micro Spin Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

9. Discard the flow through and add 200 µl *EcoPURE* Micro Wash Buffer 2 to *EcoPURE* Micro Spin Column at maximum speed for 2 minutes to completely remove any residual wash buffer.
10. Transfer the *EcoPURE* Micro Spin Column to a clean RNase-free 1.5 mL microcentrifuge tube (not provided).
11. Add 10-20 µL of *EcoPURE* Micro Elution Buffer to the center of the *EcoPURE* Micro Spin Column membrane and incubate the column at room temperature for 1 minute.
12. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.
13. Discard the *EcoPURE* Micro Spin Column and store the purified RNA at -20°C (for a few days) or -80°C (for long term storage) until use.

Protocol for Tissue Total RNA

Each isolation procedure is suitable for isolation of total RNA from up to 3-5 mg fresh or frozen tissue.

1a. Cut the tissue sample and determine the amount of tissue by weighing. Grind the sample in a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw. Immediately, add 400 µl *EcoPURE* Micro Lysis/Binding Buffer to grinded tissue. Transfer the grinded and homogenized tissue sample into a RNase-free 1.5 ml microcentrifuge tube (not provided). Mix well by vortexing for 10-15 seconds.

1b. Cut the tissue sample and determine the amount of tissue by weighing. Transfer the tissue sample into a RNase-free 1.5 ml microcentrifuge tube (not provided). Add 400 µl *EcoPURE* Micro Lysis/Binding Buffer. Immediately and vigorously homogenize using a conventional rotor-stator homogenizer with a stainless steel probe at 15,000 rpm for 30 seconds.

Precaution: *EcoPURE* Micro Lysis/Binding Buffer has the ability to inactivate the RNases in cells, however, 4 µl β-mercaptoethanol might be added to each 400 µl *EcoPURE* Micro Lysis/Binding Buffer for tissues known to have high RNase activity to ensure the inactivation of RNases.

2. Spin the lysate for 2 minutes at maximum speed in a tabletop microcentrifuge at room temperature to pellet any debris. Transfer the supernatant to another 1.5 ml RNase-free microcentrifuge tube. Note the volume of the supernatant.

3. Add equal amount of absolute (96-100%) ethanol to the supernatant and mix well by vortexing for 10 seconds.

4. Insert an *EcoPURE* Micro Spin Column into a Collection Tube and transfer 700 µl sample from step 3 to the *EcoPURE* Micro Spin Columns. Centrifuge at maximum speed in a tabletop microcentrifuge 30 sec at room temperature. Depending on your lysate volume, repeat Step 4 as necessary.

Optional: *EcoPURE* Total RNA Micro Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional On-Column DNA Removal might be applied for maximum removal of residual DNA that may affect sensitive downstream applications.

5. Discard the flow through and add 400 µl *EcoPURE* Micro Wash Buffer 1 to the *EcoPURE* Micro Spin Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

6. Discard the flow through and add 500 µl *EcoPURE* Micro Wash Buffer 2 to the *EcoPURE* Micro Spin Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

7. Discard the flow through and add 200 µl *EcoPURE* Micro Wash Buffer 2 to *EcoPURE* Micro Spin Column at maximum speed for 2 minutes to completely remove any residual wash buffer.
8. Transfer the *EcoPURE* Micro Spin Column to a clean RNase-free 1.5 mL microcentrifuge tube (not provided).
9. Add 10-20 µL of *EcoPURE* Micro Elution Buffer to the center of the *EcoPURE* Micro Spin Column membrane and incubate the column at room temperature for 1 minute.
10. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.
11. Discard the *EcoPURE* Micro Spin Column and store the purified RNA at -20°C (for a few days) or -80°C (for long term storage) until use.