Quick Start Guide

GenElute™ 96 well Total RNA Purification Kit

RTN9604

Reagents to Prepare

- Add 360 and 150 mL of 96-100% ethanol to each bottle of RNA Wash Buffer 2 and RNA Wash Buffer 3, respectively. After each use, tightly cap to prevent ethanol evaporation.
- Reconstitute RNase-free rDNase by adding 540 µL of RNase-free Water and incubate for 1 min at room temperature. Gently mix to ensure complete dissolution. If not using the entire 96 well plate, aliquot reconstituted rDNase solution and store at -20 °C.
- For each sample to be processed mix 10 µL of reconstituted rDNase with 90 µL of Reaction Buffer for rDNase.

Protocol

All centrifugations carried out at 5,600-6,000 x g.

Harvest and Lyse Pelleted Cells

1. Pellet cells by spinning for 5 min at 500 x g and remove supernatant. Add 300 µL RNA Lysis Buffer and 3 µL β-mercaptoethanol (2-ME) to each sample and mix thoroughly. Continue to **Sample Loading**.

Harvest and Lyse Attached Cells

1. Aspirate culture medium and add 130 µL RNA Lysis Buffer and 1.3 µL β-mercaptoethanol (2-ME) to each well of cell culture plate and mix thoroughly. Continue to **Sample Loading**.

Harvest and Lyse Tissue

1. Quickly slice and weigh tissue. Transfer to appropriate vessel for homogenization. Add 300 µL RNA Lysis Solution and 3 µL β-mercaptoethanol (2-ME) and homogenize until no visible pieces remain. Continue to **Sample Loading**.

Sample Loading

2. Add an equal volume (either 300 µL or 130 µL) of RNA Wash Buffer 3 and pipet up and down at least 10-15 times.
3. Place RNA Binding Plate on Square-well Block and transfer lysates to the RNA Binding Plate. Spin for 2 min and empty Square-well Block.

Bind DNA

4. Add 500 µL RNA Wash Buffer 2 to each well. Place Binding Plate on top of Square-well Block, spin for 2 min, and empty Square-well Block.
5. Carefully add 95 µL of the prepared rDNase directly to entire membrane and incubate for 15 min at room temperature.

Wash to remove contaminants

6. Add 500 µL RNA Wash Buffer 1. Place Binding Plate on top of Square-well Block, spin for 2 min, and empty Square-well Block.
7. Add 800 µL RNA Wash Buffer 2. Place Binding Plate on top of Square-well Block, spin for 2 min, and empty Square-well Block.
8. Add 500 µL RNA Wash Buffer 3. Place Binding Plate on top of Square-well Block, spin for 2 min, and empty Square-well Block.
9. Place Binding Plate on top of Square-well Block and spin for an additional 10 min to eliminate any trace ethanol. Empty Square-well Block.

Elute purified DNA

10. Place Binding Plate on top of Round-well Block and add 75 µL RNase-free water to the bottom of each well. Incubate at room temperature for 2 min and spin for 3 min to elute RNA.

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