Print only page 2-3 for Customers

Product Information Sheet

Product Name: EB Pure Total RNA Kit *

Concentration: Not Applicable

Storage and Handling:

Store at room temperature upon arrival. During shipment, crystals or precipitation may form on the TRK Buffer. Dissolve by warming buffer to 37°C.

Ordering Information:

Item Number	Preparations	Number of Tubes and Volumes
EB-TRK-50	50	EB-TRK DEPC Water: 1x10mL, EB-TRK RNA Wash Buffer I: 1x50mL, EB-TRK RNA Wash Buffer II: 1x12mL, EB-TRK TRK Lysis Buffer: 1x40mL, EB-TRK 2mL Collection Tubes: 100, EB-TRK RNA Mini Columns:50
EB-TRK-200	200	EB-TRK DEPC Water: 1x40mL, EB-TRK RNA Wash Buffer I: 1x200mL, EB-TRK RNA Wash Buffer II: 1x50mL, EB-TRK TRK Lysis Buffer: 1x150mL, EB-TRK 2mL Collection Tubes: 400, EB-TRK RNA Mini Columns: 200

Product Description:

EB Pure Total RNA Kit is a convenient system for the fast and reliable purification of RNA from cells or soft tissue. The key to this system is the High-Bind matrix that specifically, but reversibly, binds RNA under optimized conditions allowing proteins and other contaminants to be removed. Then RNA is easily eluted with deionized water or a low salt buffer. Each column can bind up to 100µg of RNA. This kit is used to recover RNA ready for applications such as RT-PCR, Northern blotting, poly A+ RNA (mRNA) purification, nuclease protection, and in vitro translation.

Additional Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000 x g
- RNase-free pipettes and 1.5 mL microcentrifuge tubes
- 100% ethanol
- 70% ethanol in sterile DEPC-treated water
- Homogenization equipment
  - Homogenizer columns
  - Needle and Syringe
  - Mortar and pestle
  - Glass beads
  - Rotor-stator Homogenizer
- Optional: 14.3M 2-mercaptoethanol (β-mercaptoethanol)

Preparation Procedure:

Prepare RNA Wash Buffer II according to the table below and store are room temperature:

- For EB-TRK-50 Kits add 48mL of 100% Ethanol to RNA Wash Buffer II Bottle and indicate addition on the bottle.
- For EB-TRK-200 Kits add 200mL of 100% Ethanol to each RNA Wash Buffer II Bottle and indicate addition on the bottle.
- Optional: Add 20 µL 2-mercaptoethanol (β-mercaptoethanol) per 1 mL TRK Lysis Buffer. This mixture can be stored for 2 weeks at room temperature.

Procedure Options:

Centrifugation Protocol – Listed below and in Lab Manual available online at empiricalbioscience.com
Vacuum Protocol – Listed in the Lab Manual available online at empiricalbioscience.com

Centrifugation Protocol

☐ 1. **For Tissue:** Determine the proper amount of starting material.
- Homogenize and disrupt the tissue.
- Add 350 µL of TRK Lysis Buffer for less than 15 mg of tissue, or 700 µL for 20-30 mg of tissue.
- Centrifuge and maximum speed (≥12,000 x *g*) for 5 minutes.
- Transfer only the clarified supernatant to a fresh microcentrifuge tube. Avoid any fatty upper layer that may have formed.

For Cultured Cells:
- Determine the proper amount of starting material.
- Harvest the cells and resuspend the pellet in 350 µL of TRK Lysis Buffer for less than 5 x 10^6 cells, or 700 µL for 5-10 x 10^6 cells.
- Homogenize the cells using a syringe and needle or homogenizer mini-columns.

Optional: Add 20 µL β-ME per 1mL TRK Lysis Buffer before using.

☐ 2. Add 1 volume 70% ethanol. Vortex to mix thoroughly. Do not centrifuge. If the sample has lost any volume during homogenization, adjust the volume of ethanol to match.

☐ 3. Insert a High-Bind RNA Mini Column into a 2mL Collection Tube (provided).

☐ 4. Transfer 700 µL sample to the Mini Column. Centrifuge at 10,000 x *g* for 1 minute. Discard the filtrate and reuse the Collection Tube.

☐ 5. Repeat Step 4 until all of the sample has been transferred to the column.

☐ 6. **Optional:** Perform DNase digestion according to your preferred protocol.

☐ 7. Add 500 µL RNA Wash Buffer I to the column. Centrifuge 10,000 x *g* for 30 seconds. Discard the filtrate and reuse the Collection Tube.

☐ 8. Add 500 µL RNA Wash Buffer II diluted with 100% ethanol (See Bottle) to the column. Centrifuge 10,000 x *g* for 1 minute. Discard the filtrate and reuse the Collection Tube.

☐ 9. Repeat Step 8.

☐ 10. Centrifuge at maximum speed for 2 minutes to dry the column.

☐ 11. Transfer the High-Bind Column to a clean 1.5mL microcentrifuge tube (not provided).

☐ 12. Add 40-70 µL DEPC Water. Centrifuge at maximum speed for 2 minutes.

☐ 13. Store eluted RNA at -70°C.