

Product Information Sheet and Protocol

Product Name: EB Pure Total RNA Kit*

Item No.:

| Item Number | Preparations |
|-------------|--------------|
| SS-EB-TRK-5 | 5 |
| EB-TRK-50 | 50 |
| EB-TRK-200 | 200 |

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.

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.

Kit contents:

| Component | SS-EB-TRK-5 | EB-TRK-50 | EB-TRK-200 |
|----------------------|-------------|-----------|------------|
| RNA Mini Columns | 5 | 50 | 200 |
| 2mL Collection Tubes | 10 | 100 | 400 |
| TRK Lysis Buffer | 5mL | 40mL | 150mL |
| DEPC Water | 1.5mL | 10mL | 40mL |
| RNA Wash Buffer I | 5mL | 50mL | 200mL |
| RNA Wash Buffer II | 5mL | 12mL | 50mL |

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 at room temperature:

| Kit | 100% Ethanol to be Added |
|-------------|--------------------------|
| SS-EB-TRK-5 | 20mL |
| EB-TRK-50 | 48mL |
| EB- TRK-200 | 200mL per 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.

* This product is for “Research Use Only. Not for use in diagnostic procedures”.

For MSDS and Certificate of Analysis please visit www.empiricalbioscience.com

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 at maximum speed ($\geq 12,000 \times g$) 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 5×10^6 cells, or 700 μL for $5-10 \times 10^6$ 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 $\times g$ 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 $\times g$ 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 $\times g$ 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 .

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