rNEAT™ Tissue Total RNA Purification Kit

PURK-RNA-100

Description

The rNEAT™ Tissue Total RNA Purification Kit offers a rapid and convenient method for purification of total RNA from a variety of tissue and culture cells. The kit is based in the nucleic acid ability to bind silica membranes in the presence of high concentrations of chaotropic salts. Tissue samples can be efficiently homogenized in a microcentrifuge tube using the provided micropestle. The purified RNA is then ready for its use in a variety of applications: real-time RT-PCR, Northern Blotting, cDNA library construction, etc.

Features

- ✓ High yields: up to 50µg; depends on type of sample
- ✓ Ready to use RNA
- ✓ Just a few minutes procedure (about 30 min)
- ✓ Mini format

Kit Components	50 rxn
Buffer BLY*	25 ml
Wash Buffer 1 (WB1)	30 ml
Wash Buffer 2 ** (WB2)	15 ml
RNase-free ddH2O	10 ml
rNEAT™ spin column	50
Filter Column	50
Collection tube (2mL)	10
1.5 ml microtube	50
Micropestle	50

Note

*Before beginning the lysis and homogenization steps, prepare a fresh amount of Buffer BLY containing 1% 2-mercaptoethanol (β-ME) [Not included] for each purification procedure. Add 10 µl β-ME for each 1 mL Lysis Buffer (Buffer BLY).

**Add the volume ethanol (96%-100%) specified [Not included] to WB2 Buffer prior to initial use (see bottle label for volume). After ethanol has been added, mark the bottle to indicate that this step has been completed.

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing.

Quality Certifications

Total RNA is isolated from a 30 mg thorax muscle tissue sample. Purified RNA is quantified using a spectrophotometer with a typical yield of more than 10µg of total RNA and a A260nm/A280nm ratio of 1.9-2.1. Quality is further checked by agarose gel electrophoresis.

Product use limitation

This product is developed, designed, and sold exclusively for research purposes and use only. The product is not intended for diagnostics or drug development, nor is it suitable for administration to humans or animals.

Kit Storage

Can be stored at room temperature. The kit components are stable for 1 year, if stored properly.

Protocol

1. Cut up to 30mg of animal tissue and transfer to a 1.5-ml microcentrifuge tube (not provided). Use the **micropestle** to grind the material to pulp (or a motor-driven grinder). You can grind the tissue sample in liquid nitrogen. Add additional 200µl of Buffer BLY (β-ME added) and continue to homogenize the sample by vortex 15-30 sec.
2. Add 350µl of **Buffer BLY** (β-ME added) and continue to homogenize the sample by grinding. To release all RNA in the sample, it is required to disrupt the sample completely.
3. Incubate at room temperature for 5 minutes.
4. Place a **Filter Column** in a 2 ml **Collection tube** and transfer the sample mixture to the filter column. Centrifuge at 10,000 g for 2 minutes.
5. Carefully transfer the clarified filtrate to a new 1.5 ml microcentrifuge tube.
6. Add 1 volume of 70% ethanol to the clarified lysate and mix vigorously by vortexing.
7. Apply the total volume (usually 700 µl) from step 6 to the **rNEAT™ spin column** by decanting or pipetting
8. Centrifuge at 10,000 g for 90 seconds. Discard the flow-through.
9. [Optional] To eliminate genomic DNA contamination, follow the steps from a) to d). Otherwise, proceed to step 10 directly:

   a) Add 250 µl of **WB1** to wash **rNEAT™ spin column**. Centrifuge at full speed for 1 min
   then discard the flow-through.

   b) Add 60 µl of RNase-free DNase 1 solution (0.5U/µl, not provided) to the membrane centre of **rNEAT™ spin column**. Let stand for 15 minutes at room temperature.

   c) Add 250 µl of **WB1** 1 to wash **rNEAT™ spin column**. Centrifuge at full speed for 1 min then discard the flow-through.

   d) After DNase 1 treatment, proceed to step 11.
10. Add 500 µl of **WB1** and centrifuge at full speed for 30 seconds. Discard the flowthrough.
11. Add 750 µl of **WB2** and centrifuge at full speed for 30 seconds. Discard the flowthrough.
12. Again, Centrifuge at full speed for 2 minutes. This step helps to dry the **rNEAT™ spin column**.
13. Place the **rNEAT™ spin column** into a new, labelled 1.5 microcentrifuge tube and pipet 40-50µl of **Elution buffer** directly into the centre of the column membrane. Close the cap and incubate for 1 minute at room temperature.
14. Centrifuge at full speed for 1 minute to elute RNA.
15. Always keep eluted RNA on ice and store at <-70°C.