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EasyRNA™ Bacterial RNA Mini Kit

(Cat# K1351-50, -250; Store at Multiple Temperatures)

I. Introduction:

BioVision's EasyRNA™ Bacterial RNA Mini Kit provides an easy and fast method for isolating total RNA from total RNA from Gram-positive (*B. subtilis*) Or Gram-negative (*E. coli*) Bacteria within 30 min. Only trace genomic DNA exists in the purified RNA, which can be eliminated by DNase I treatment (See detail in the protocol) when it is necessary.

II. Sample Type: For fast and efficient isolation of total RNA from plant tissues within 30 min.

III. Kit Contents:

Components	K1351-50 50 Preparations	K1351-250 250 Preparations	Part Number
Buffer LY	28 mL	135 mL	K1351-XX-1
Buffer RB	30 mL	135 mL	K1351-XX-2
RNA Wash Buffer*	12 mL	3 x 50 mL	K1351-XX-3
DEPC-Treated ddH_2O	10 mL	30 mL	K1351-XX-4
RNA Columns	50	250	K1351-XX-5
Lysozyme	15 mg	75 mg	K1351-XX-6

**Add 80 mL (K1351-50) or 96 mL (K1351-250) 100% ethanol to RNA Wash Buffer before use. **Add 9.6 mL (K1351-50) or 48 mL (K1351-250) 100% ethanol to DNase Stop Buffer before use.*

IV. User Supplied Reagents and Equipment:

- Tabletop microcentrifuge and 1.5 mL sterile tubes; Vacuum manifold if use vacuum protocol; 100% ethanol; β-mercaptoethanol; ***Optional: DNase I, DNase Buffer***

V. Shipment and Storage:

All the reagents are shipped at room temperature. DNase I (optional) and lysozyme should be stored at -20°C. All other components can be stored at room temperature. All kit components are guaranteed for 12 months from the date of purchasing.

VI. Reagent Preparation and Storage Conditions:

- Prepare all components and get all necessary materials ready by examining the protocol and become familiar with each step.
- Add 1% volume of β-mercaptoethanol to Buffer LY before use and store at 4°C.
- Add 80 mL (K1351-50) or 96 mL (K1351-250) 100% ethanol to RNA Wash Buffer before use.
- Add 9.6 mL (K1351-50) or 48 mL (K1351-250) 100% ethanol to DNase Stop Buffer before use. The final ethanol is 80% (v/v).
- Prepare a lysozyme stock solution at 3 mg/mL or 0.4 mg/mL with Elution Buffer or TE Buffer and aliquot into adequate portions. Store each aliquot at -20℃ and thaw before use.

VII. <u>Protocol for Extracting Total RNA From Gram positive (*B. subtilis*) Or Gram-negative (*E. coli*) Bacteria</u>

1. Grow an overnight bacterial culture in the appropriate media at an appropriate temperature. In the following day, dilute the culture 1:50 with media and grow until the OD_{600} reads at 0.6-1.0. This should only take a few hours. If the growth is too slow, reduce the dilution factor. Do not use the overnight culture for RNA isolation!
2. Harvest no more than **3 mL culture** (<$5x10^8$) by centrifugation at 3,000 rpm for 10 min.
3. Carefully remove the supernatant as much as possible.
4. Resuspend the pellet in **100 µL freshly prepared TE Buffer** (10 mM, TrisHCl, pH 8.0; 1mM EDTA, pH 8.0) or **Elution Buffer** (10 mM Tris-HCl, pH 8.5) containing lysozyme. (Use 3 mg lysozyme per 1 mL TE Buffer for Gram-positive bacteria and 0.4 mg/mL lysozyme for Gram-negative bacteria). Mix by tapping gently.
5. Incubate the resuspended pellet at room temperature for 5-10 min for Gram-positive bacteria, or 3-5 min for Gram-negative bacteria.
6. Add **400 µL Buffer LY**. Mix gently. Centrifugation at 13,000 rpm for 5 min, transfer the clear lysate to a new RNase-free tube. Add **0.5 volume 100% ethanol** to the lysate (For example: 250 µL 100% ethanol for 500 µL lysate). Ensure that β-mercaptoethanol has been added before use.
7. Transfer the solution into the RNA column and centrifuge at 12,000 rpm for 1 min. Discard the collection tube with the flow-through and put the column back to a new collection tube.
8. Add **500 µL Buffer RB** to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through.
9. Add another **500 µL RNA Wash Buffer** to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through. and put the column back to the collection tube.
10. Centrifuge the column at 12,000 rpm, with the lid open, for another 1 min. It is critical to remove residual ethanol for optimal elution.
12. Place the column to a RNase-free 1.5 mL tube, add **50-100 µL DEPC treated ddH_2O** to the column and centrifuge at 13,000 rpm for 2 min. The RNA is in the flow-through liquid. Store the RNA solution at -20℃.

Note: It is highly recommended that RNA quality be determined before downstream applications. The quality of RNA can be assessed by denatured agarose gel electrophoresis with the ethidium bromide staining. Several sharp bands should appear on the gel including 28S and 18S ribosomal RNA bands as well as certain populations of mRNA and bands. If these bands smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling or storage, RNA molecule less than 200 bases in length do not efficiently bind to the RNA column. An A_{260}/A_{280} ratio of 1.8-2.0 corresponds to 90-100% pure nucleic acid.

FOR RESEARCH USE ONLY! Not to be used on humans.