

Hieff™ Bacterial RNA Kit

Product description

This Kit is designed for rapid and efficient RNA extraction from Gram-positive and Gram-negative bacteria. Utilizing a proprietary silica- membrane column technology combined with optimized buffers, this kit effectively removes contaminants such as proteins, metabolites, and genomic DNA. The purified RNA is suitable for downstream applications including RT-PCR, RT-qPCR, in vitro transcription, and molecular cloning.

Specifications

Cat.No.	19301ES50
Size	50 T

Components

Category	Components No.	Name	19301ES50
Part I	19301-A	Lysozyme	20 mg
Part II	19301-B	DNA-Removing Column B4	50 units
	19301-C	Hieff™ RNA Column B4	50 units
	19301-D	2 mL Collection Tube B4	100 units
	19301-E	Lysis Buffer(LB Buffer B4)	25 mL
	19301-F	PL Buffer B4	40 mL
	19301-G	Wash Buffer B4*	13 mL(add 52 mL ethanol before use)
	19301-H	TE Buffer pH 8.0	6 mL
	19301-I	RNase-free H ₂ O	5 mL

Storage

Part I components should be stored at 4~8°C for 2 years.

Part II components should be stored at room temperature for 2 years.

Notes

1. Wear lab coats and gloves. Avoid RNase contamination.
2. Ensure buffers are tightly closed to prevent evaporation, oxidation, or pH changes.
3. Inspect solutions for precipitation or turbidity, particularly in cold environments(e.g., winter). If observed, warm the solutions at 37°C until clear to ensure optimal performance.
4. For research use only.

Instructions

Pre-Experiment Preparation

- 1) **Required equipment and reagents:** tabletop centrifuge, shaker, water bath or metal bath, chloroform, absolute ethanol, 1.5 mL RNase-free centrifuge tubes, etc.
- 2) **Centrifugation Conditions:** All centrifugation steps should be performed at room temperature using a standard benchtop centrifuge capable of reaching 12,000 rpm.
- 3) **Wash Buffer B4 Preparation*:** Before the first use, add 4 times the volumes of anhydrous ethanol (e.g., 52 mL for 13 mL of Wash Buffer B4*) to the Wash Buffer B4* bottle. Mix thoroughly and label the bottle and Seal the cap tightly after each use to maintain ethanol content.

[Note]: If the volume appears inaccurate due to transportation or storage issues, adjust to the correct volume using a measuring cylinder before adding ethanol.

- 4) **Lysozyme Working Solution Preparation:** Before first use, dissolve 1 mg of Lysozyme (19301-A, or Cat# 10402ES) in 1 mL of TE Buffer (pH 8.0) to prepare a 1 mg/mL Lysozyme working solution. Mix thoroughly, aliquot, and store at -20°C; the solution remains stable for several weeks

Procedure

1. Sample Pretreatment

- 1) Take 0.5-2 mL of bacterial culture (not exceeding 10^9 cells), Centrifuge at 12,000 rpm for 2 minutes and discard the supernatant.

[Note]: Avoid using excessive bacteria, as this will significantly reduce RNA yield. The initial volume depends on bacterial density and type.

[Note]: Ensure residual supernatant does not exceed 20 μ L.

2) Lysozyme Treatment:

- For Gram-negative bacteria: Add 100 μ L of 1 mg/mL Lysozyme working solution. Vortex for 10 seconds, incubate at room temperature for 2 minutes. Repeat vortexing and incubation three times.
- For Gram-positive bacteria: Add 100 μ L of 1 mg/mL Lysozyme working solution. Vortex for 10 seconds, incubate at room temperature for 2 minutes. Repeat vortexing and incubation six times.

[Note]: If the bacterial count exceeds 5×10^8 cells, use 200 μ L of Lysozyme working solution.

[Note]: Gram-positive bacteria cell wall disruption varies by type; optimize Lysozyme concentration and incubation conditions as needed.

- 3) Briefly centrifuge to collect the cells and discard the supernatant.

- 4) Resuspend the cells by vortexing. Add 500 μ L of LB Buffer B4(19301-E), pipette up and down to mix, and vortex vigorously until no visible insoluble material remains.

[Note]: If insoluble material persists, centrifuge at 12,000 rpm for 2 minutes and use the supernatant for the next step.

2. RNA Extraction

- 1) Place a DNA-Removing Column B4(19301-B) into a 2 mL Collection Tube B4 and set aside.

- 2) Add the lysate from step 1-4 to the DNA-Removing Column B4. Centrifuge at 12,000 rpm for 1 minute and retain the flow-through.

[Note]: If liquid remains on the membrane, extend centrifugation to 5 minutes.

3) Add 0.5 volumes of anhydrous ethanol to the flow-through and mix immediately by pipetting.

[Note]: Flocculent precipitation may form; this is normal.

4) Transfer the mixture to a Hieff™ RNA Column B4(19301-C). Centrifuge at 12,000 rpm for 1 minute and discard the waste liquid.

[Note]: The maximum volume per addition is 700 µL; perform multiple centrifugations if necessary.

5) Add 500 µL of PL Buffer B4(19301-F). Centrifuge at 12,000 rpm for 30 seconds and discard the waste liquid.

6) Place the Hieff™ RNA Column B4 back into the collection tube. Add 500 µL of Wash Buffer B4*(19301-G). Centrifuge at 12,000 rpm for 30 seconds and discard the waste liquid.

[Note]: Ensure anhydrous ethanol has been added to Wash Buffer B4*.

7) Repeat step 6.

8) Place the Hieff™ RNA Column B4 back into the collection tube. Centrifuge empty at 12,000 rpm for 2 minutes at room temperature to remove residual Wash Buffer B4*.

9) Transfer the Hieff™ RNA Column B4 to a new 1.5 mL RNase-free centrifuge tube. Add 30-50 µL of RNase-free H₂O to the center of the column. Let it stand at room temperature for 1 minute, then centrifuge at 12,000 rpm for 1 minute. Collect the flow-through, which contains the RNA solution.

[Note]: To increase recovery yield:

① Pre-warm RNase-free H₂O to 65°C.

② Reapply the RNA flow-through to the column, let it stand for 2 minutes, and elute again.

10) Store the RNA sample at -20°C for short-term storage or -80°C for long-term storage.