SPINeasy™ RNA Kit for Bacteria

Cat. No.: 116541050 (50 PREPS) / 116541000 (5 PREPS)

Quick-Start Protocol

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Notes before starting:

- Add 50 mL (5 mL for sample kit) of absolute ethanol to Wash Buffer R and mark the bottle.
- Prepare DNase I solution by spinning down briefly the vial of lyophilized DNase I provided, resuspend and dissolve with 500 µL **Nuclease-free water**. Store DNase I solution at -20 °C in aliquots and avoid repeated freeze-thawing. Do not prepare DNase I solution in DNase I Buffer.
- Use maximum centrifugation speed available if 14,000 *g* is not feasible.
- Lysis can be performed by vortexing the sample in a vial of Lysing Matrix B at the maximum speed for 5 min if a FastPrep® Instrument is unavailable.
- For RNA extraction from gram-positive bacteria (optional) : prepare pre-treatment buffer according to instruction on the back of this protocol (to be supplied by user).

Column preparation

1. Add **200 µL Equilibration Buffer R** to **Column R with collection tube** membrane to ensure its performance. Centrifuge for **1 min @ maximum speed**. Discard the flow-through and reuse the collection tube.

Lyse

2. Pellet 1 – 6 mL bacterial culture by centrifugation for **3 mins @ 10,000 *g***, discard supernatant.
3. Resuspend cell pellet in **250 µL RNASS** and transfer to a vial of **Lysing Matrix B**.
4. Add **750 µL Lysis Buffer R**.
5. Homogenize in a FastPrep® Instrument for **40 sec** at speed setting of **6.0 m/s.**

Bind

6. Centrifuge for **10 mins @ 14,000 *g*** and transfer **750 µL supernatant** to a clean, nuclease-free 2 mL microcentrifuge tube.
7. Add **1:1 volume** of **absolute ethanol** (e.g., 750 µL ethanol to 750 µL lysate supernatant) and mix well by pipetting up and down.
8. Transfer **750 µL mixture** to **Column R with collection tube.**
9. Centrifuge for **1 min @ 14,000 *g***. Discard flow through and reuse collection tube.
10. Repeat steps 8 and 9 to load the remaining mixture.

Wash

11. DNase I digestion: In a clean 1.5 mL microcentrifuge tube, add 5 µL DNase I solution to 75 µL **DNase I Buffer** per prep. Mix well and add **80 µL** to the center of the column membrane. Incubate at room temperature for **15 mins**.
12. Add **500 µL Wash Buffer R** to the column.
13. Centrifuge for **1 min @ 14,000 *g***. Discard flow through and reuse collection tube.
14. Repeat step 12 and 13 to wash a second time.
15. Centrifuge for an additional **1 min @ 14,000 *g*** to dry column. Remove collection tube and place column onto a clean 1.5 mL microcentrifuge tube.

Elute

16. Add **100 µL Nuclease-free water** to the center of the membrane. Incubate at room temperature for **1 min**. For samples with low RNA content, reducing the elution volume to **50 µL** may increase the concentration of eluted RNA.
17. Centrifuge for **1–2 min @ 8,000 *g*** to elute RNA.
18. Eluted RNA will be collected in the microcentrifuge tube. For the best results, proceed to perform downstream applications immediately and keep RNA chilled on ice while working, to prevent degradation. Store remaining RNA at -80 °C in aliquots and avoid repeated freeze-thawing.

Pre-treatment of Gram-positive Bacteria (Optional):

1. To prepare 20 mg/mL lysozyme (to be supplied by user) with Gram-positine Bacteria Pre-treatment Buffer included in the kit. Store at 4 °C after adding lysozyme.
2. Harvest cells by centrifugation at 10,000 *g* for 3 mins and discard supernatant. Resuspend bacterial pellet in 200 µL lysozyme pretreatment buffer.
3. Incubate for 30 mins at 37 °C.
4. Transfer sample to a vial of Lysing Matrix B, add 250 µL RNASS and 750µL Lysis Buffer R.
5. Proceed to step 5 of the RNA Extraction Protocol.

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