Instruction Manual

SPINeasy RNA Kit for Bacteria

(With Lysing Matrix)

Spin Column Kit for Easy Isolation of RNA from Bacteria Samples

Cat. No.:

116541050 (50 Preps)

116541000 (5 Prep Sample Kit)

Storage: 15 – 25 °C

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1. Introduction

SPINeasy RNA Kit for Bacteria is a silica-membrane spin-column kit that enables quick and convenient purification of total RNA from gram-positive and gram-negative bacteria. Included in the kit is our specially formulated RNASS solution that stabilizes and protects RNA in bacteria samples. The use of Lysis Buffer R and Lysing Matrix B in combination with FastPrep® instruments from MP Biomedicals enables highly efficient lysis of bacterial samples within seconds. With a simple workflow, the kit allows multiple samples to be processed simultaneously, without the use of toxic substances such as phenol and chloroform. Total RNA of high quality and integrity can be purified typically within 40 minutes and is immediately available for RT-PCR and other downstream applications. Visit www.mpbio.com to explore additional products to support your research.

2. Kit Components and User Supplied Materials

2.1 Kit Components

Components	Package	Cat. No.
Lysing Matrix B	50 ea	116911050
RNASS	15 mL	116541051
Lysis Buffer R	60 mL	116541052
Wash Buffer R	6 mL	116541053
Nuclease-free water	10 mL	116541054
DNase I	1 vial	116541055
DNase I Buffer	5 mL	116541056
Column R with collection tube	50 ea	116541057
Instruction Manual	1 ea	-
Quick-Start Protocol	1 ea	-
MSDS & CoA	Available www.mpbio.com	

2.2 User Supplied Materials

- FastPrep instrument - FastPrep-24™ 5G (Cat. No.116005500)
- Vortex (an adapter for multiple microcentrifuge-sized tubes is recommended if multiple samples are to be processed simultaneously), if FastPrep instrument is unavailable
- Microcentrifuge capable of at least 14,000 x g
- Absolute ethanol (50 mL for preparing Wash Buffer R and 750 µL per prep for sample preparation)
- Nuclease-free 2 mL microcentrifuge tubes
- Nuclease-free 1.5 mL microcentrifuge tubes
- Optional: Gram-positive bacteria pre-treatment buffer containing 20 mM Tris-HCl pH 8.0, 2 mM EDTA pH 8.0, 1.2% Triton® X-100 and 20 mg/mL lysozyme, prepared with RNase-free water. Store at 4 °C after adding lysozyme.

3. Storage and Stability

All SPINeasy RNA Kit for Bacteria components are guaranteed for at least 24 months from the date of manufacture when stored at room temperature (15 – 25 °C).

4. Notes Before Starting

Please check as appropriate:

- Add 50 mL (5 mL for sample kit) of absolute ethanol to Wash Buffer R and mark the bottle.
- Prepare DNase I solution according to instructions in Section 6.1.
- Prepare one 2 mL microcentrifuge tube per prep for lysate binding preparation.
- Prepare one 1.5 mL microcentrifuge tube per prep for elution of purified RNA.
- Prepare one 1.5 mL microcentrifuge tube for DNase I-buffer mixture.
- Lysis can be performed by vortexing the sample in a vial of Lysing Matrix B at the maximum speed if a FastPrep instrument is unavailable.
- Centrifugation speed stated in the manual will be a guideline; use the maximum speed available if 14,000 x g is not feasible.

5. Safety Precautions

Lysis Buffer R contains a component that can be harmful if swallowed and may cause irritation when in contact with skin and eyes. To prevent accidental ingestion, do not eat, drink or smoke when using this product. Wear personal protective equipment (gloves, lab coat and eye protection) to prevent contact with the skin or mucous membranes. Consult the Material Safety Data Sheet at www.mpbio.com for additional details.

6. Protocol

A Quick-Start Protocol is provided in the kit for quick reference throughout the extraction process.

6.1 Preparation of DNase I solution

Briefly spin down the vial of lyophilized DNase I provided and resuspend with 500 µL of **Nuclease-free water**. Mix well to dissolve. Store DNase I solution at -20 °C in aliquots and avoid repeated freeze-thawing.

Note: Do not prepare DNase I solution in DNase I Buffer.

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

If necessary, pre-treatment of gram-positive bacteria with lysozyme may be performed, which may result in higher RNA yields. Good quality RNA of reasonable yields can also be obtained without performing the pre-treatment.

1. Prepare lysozyme pre-treatment buffer (to be supplied by user) with the composition outlined below. The use of nuclease-free water is strongly recommended for preparing this buffer.
   - 20 mM Tris-HCl, pH 8.0
   - 2 mM EDTA, pH 8.0
   - 1.2% Triton® X-100
   - 20 mg/mL lysozyme. (Store at 4 °C after adding lysozyme.)
2. Harvest cells by centrifugation at 10,000 x g for 3 mins and discard supernatant. Resuspend bacterial pellet in 200 µL of lysozyme pre-treatment buffer.
3. Incubate for 30 mins at 37 °C.
4. Transfer sample to a vial of **Lysing Matrix B**, add 250 µL of **RNASS** and 750 µL of **Lysis Buffer R**.
5. Proceed to step 4 of the RNA Extraction Protocol.
6. Note that in this case, up to 950 µL of the lysate supernatant may be used in step 6 of the RNA Extraction Protocol. Consequently, add 1:1 volume of ethanol proportionately and repeat sample loading steps (steps 8 and 9) to load all the mixture.

6.3 RNA Extraction Protocol

1. Pellet 1 – 6 mL bacterial culture by centrifugation at 10,000 x g for 3 mins and discard supernatant.
2. Resuspend cell pellet in 250 µL of **RNASS** and transfer to a vial of **Lysing Matrix B**.
3. Add 750 µL of **Lysis Buffer R**.
4. Homogenize in a FastPrep instrument for 40 seconds at speed setting of 6.0 m/s.
   Alternatively, vortex samples at the maximum speed for 5 mins if a FastPrep instrument is not available. It is recommended to perform vortexing with the use of an adapter to hold the vials, especially if multiple samples are to be processed simultaneously.
5. Centrifuge at 14,000 x g for 10 mins.

Note: Centrifuge at the maximum speed for all steps if 14,000 x g is not feasible.

6. Carefully pipette out 750 µL of the 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 of the mixture to a **Column R with collection tube**.
9. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube.
10. Repeat steps 8 and 9 to load the remaining mixture.
11. DNase I digestion:

    In a clean 1.5 mL microcentrifuge tube, add 5 µL of DNase I solution to 75 µL of **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.

    Note: Mixture of DNase I solution with DNase I buffer should be freshly prepared prior to usage. It is not recommended to store the remaining mixture.
12. Add 500 µL of **Wash Buffer R** to the column.
13. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube.
14. Add 500 µL of **Wash Buffer R** to the column.
15. Centrifuge at 14,000 x g for 1 min. Discard flow through and reuse collection tube.
16. Centrifuge at 14,000 x g for an additional 1 min to dry column.
17. Remove collection tube and place column onto a clean 1.5 mL microcentrifuge tube.
18. Add 100 µL of **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.
19. Centrifuge at 8,000 x g for 1 – 2 mins to elute RNA.
20. Eluted RNA will be collected in the microcentrifuge tube. For 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.

7. Data

The following results demonstrate examples of RNA extracted from bacteria samples using SPINeasy RNA Kit for Bacteria. Extracted RNA are of high yield, quality and integrity, as indicated by concentration readings, A_{260}/A_{280} and A_{260}/A_{230} ratios of around 2, and 23S and 16S bands clearly resolved in agarose gel electrophoresis. RNA extracted with this kit can be amplified by RT-PCR.

Table 1: Quality and quantity of RNA extracted from representative bacteria samples using SPINeasy RNA Kit for Bacteria.

Sample	Starting Amount	Extraction Results		
		Concentration (ng/µl)	$A_{260/280}$	$A_{260/230}$
E. coli	Pellet from 3 mL overnight culture	763.59	2.10	2.26
S. aureus	Pellet from 3 mL overnight culture	349.47	2.13	2.04

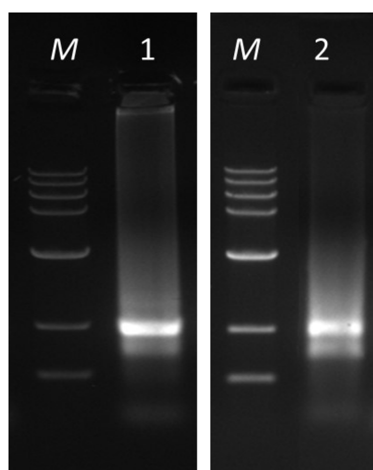

Figure 1: Total RNA extracted from bacteria samples using SPINeasy RNA Kit for Bacteria, analyzed using agarose gel electrophoresis. *M*: DNA marker; Lane 1: *E. coli*; Lane 2: *S. aureus*.

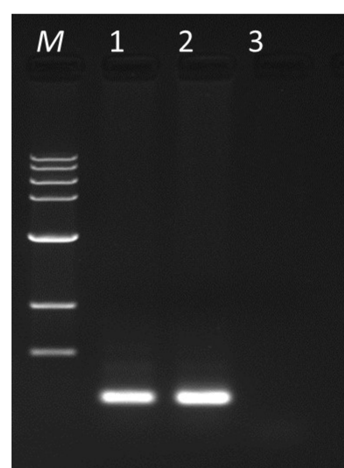

Figure 2: RT-PCR amplification of RNA extracted from bacteria samples using SPINeasy RNA Kit for Bacteria. *M*: DNA marker; Lane 1: *E. coli*; Lane 2: *S. aureus*; Lane 3: Negative control.

8. Troubleshooting

8.1 Low/ No RNA Yield

1. Ensure the extraction was performed according to kit manual instructions.
2. Sample with low RNA content: (i) Increase amount of starting material; (ii) Elute in a smaller volume (50 µL).
3. Insufficient lysis or over-lysis: When using a vortex instead of a FastPrep for sample homogenization, lysis condition may be optimized by testing reduced or extended vortexing duration.
4. For gram-positive bacteria, performing pre-treatment with lysozyme can lead to increased RNA yields (Refer to 6.2).
5. Sample incompatible with RNASS. While RNA yield and quality are greatly enhanced with the use of RNASS for most bacteria samples, RNASS may not be suitable for use with certain sample types. In these cases, resuspend bacterial pellet with 1 mL of Lysis Buffer R, transfer to Lysing Matrix B and proceed to step 4 of the RNA Extraction Protocol without using RNASS.
6. Poor sample quality. For best results, freshly prepared samples should be used.
7. RNase contamination. Work with nuclease-free tubes and pipette tips. Handle samples and perform all steps with clean gloves. Decontaminate work surfaces with RNase Erase® (Cat. No. 112440204).

8.2 Smeared RNA Bands

1. Poor sample quality. For best results, freshly prepared samples should be used.
2. Sample over-lysis. Reduce FastPrep speed and/or duration.
3. RNA degradation. Work with freshly purified RNA and keep RNA chilled on ice after elution. RNA should be stored at -80 °C and freeze-thawing should be avoided.

8.3 DNA Contamination

1. Perform on-column DNase I digestion according to step 11 of the RNA extraction protocol. Ensure that DNase I solution is prepared according to manual instructions. Once dissolved, DNase I should be stored at -20 °C.

9. Product Use Limitations & Warranty

The products presented in this instruction manual are for research or further manufacturing use only. They are not to be used as drugs or medical devices to diagnose, cure, mitigate, treat or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery. Buyer's exclusive remedy and the sole liability of MP Biomedicals hereunder shall be limited to, at our discretion, no replacement or compensation, product credits, refund of the purchase price of, or the replacement of materials that do not meet our specification. By acceptance of the product, Buyer indemnifies and holds MP Biomedicals harmless against, and assumes all liability for, the consequence of its use or misuse by the Buyer, its employees or others, including, but not limited to, the cost of handling. Said refund or replacement is conditioned on Buyer notifying within thirty (30) days of receipt of product. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s). MP®, GENECLEAN®, FastPrep®, and 7X® are registered trademarks of MP Biomedicals, LLC.

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