USER GUIDE

AccuPrep®
Bacterial RNA Extraction Kit

Cat. No. K-3142
K-3143

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AccuPrep® Bacterial RNA Extraction Kit

Kit for the extraction of RNA from bacterial cultures

User Guide

K-3143 Σ **50**

K-3142 Σ **100**

Version No.: 2 (2022-03-22)

Please read all the information in booklet before using the unit

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Intended Use

AccuPrep® Bacterial RNA Extraction Kit is developed and supplied for research purposes only. Certain applications possible with this kit may require special approval by appropriate local and/or national regulatory authorities in the country of use.

Safety Warning and Precaution

Wear appropriate protection when handling any irritant or harmful reagents. The use of a laboratory coat, protective gloves and safety goggles are highly recommended. For more information, please consult the appropriate Material Safety Data Sheet (MSDS).

Warranty and Liability

All BIONEER products undergo extensive Quality Control testing and validation. BIONEER guarantees quality during the warranty period as specified, when following the appropriate protocol as supplied with the product. It is the responsibility of the purchaser to determine the suitability of the product for its particular use. Liability is conditional upon the customer providing full details of the problem to BIONEER within 30 days.

Quality Management System ISO 9001 Certified

Every aspect of our quality management system from product development, production to quality assurance and supplier qualification meets the world-class standards.

Trademark

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Contents

Product Information

Components

Components	K-3143 (50 reactions)	K-3142 (100 reactions)	Storage
Proteinase K powder, lyophilized	25 mg	25 mg x 2 ea	Refer to the "Storage" below.
RS Buffer (Stabilizing)	40 ml	80 ml	Store at room temperature (15-25°C).
RB Buffer (Binding)	60 ml	90 ml	
RWA1 Buffer (1st Washing)	40 ml	80 ml	
RWA2 Buffer (2nd Washing)	70 ml	70 ml x 2 ea	
ER Buffer (Elution)	10 ml	20 ml	
AccuPrep® Binding Column-III	50 ea	100 ea	
1.5 ml Tube (Elution)	50 ea	100 ea	
One Page Protocol	1 ea	1 ea	

Storage

The kit will maintain performance for at least two years under standard storage conditions.

All components of the kit should be stored dry at room temperature (15-25°C). Before use, lyophilized Proteinase K should be completely dissolved in 1,250 µl of nuclease-free water. For short term storage, dissolved Proteinase K should be stored at 4°C. For long term storage, it is recommended to aliquot the enzyme into separate tubes and store at -20°C.

*** Note:** Please note that repeated freezing and thawing may reduce its activity.

Specifications

AccuPrep® Bacterial RNA Extraction Kit		
Amount of Starting Sample		1 x 10^8 cells
Turnaround Time		< 40 min
Column Binding Capacity		Up to 120 µg
Elution Volume		30-100 µl
Typical RNA Yield	Gram-negative bacteria	4-8 µg
	Gram-positive bacteria	3-6 µg
RNA Purity		A_{260}/A_{280} > 2.0, A_{260}/A_{230} > 1.7
Isolation Technology		Silica Column

*** Note:** There may be differences in measured values depending on the type of samples.

Precautions

- Take appropriate laboratory safety precautions and wear gloves when handling because RB Buffer and RWA1 Buffer contain chaotropic salts which are irritants.
- RNA is highly susceptible to degradation by exogenous RNase that may be introduced during the handling steps, all the steps must be conducted under sterile, RNase-free condition.
- RNase-free reagents, pipette tips, and tubes must be used with gloved hands while handling them.

Introduction

Product Description

AccuPrep® Bacterial RNA Extraction Kit is designed for extraction of highly purified RNA from gram-positive and gram-negative bacterial within 40 minutes. The kit employs *AccuPrep*® Binding Column-III with silica membrane for nucleic acid binding in the presence of chaotropic salts.

Pelleted bacterial cells are resuspended with a guanidine-thiocyanate-containing buffer, which is a highly denaturing agent and inactivates RNase to isolate RNA. Samples are applied to the *AccuPrep*® Binding Column-III which binds total RNA. Cell debris and other contaminants are eliminated by subsequent washing, and highly purified RNA is eluted in elution buffer or RNase-free water. The process does not require phenol/chloroform extraction, ethanol precipitation, or other time-consuming steps.

RNA extracted through this kit can be used for a variety of applications, including: reverse transcription PCR (RT-PCR), reverse transcription quantitative PCR (RT-qPCR), northern blot analysis, and cDNA synthesis. We recommend DNase treatment for only RNA quantitation.

Features & Benefits

- Comprehensive: High quality and yield of RNA extraction from gram-positive and gram-negative bacteria.
- Convenient & Rapid: The process does not require phenol/chloroform extraction, ethanol precipitation, or other time-consuming steps.
- Ready-to-use: Extracted RNA is ready-to-use for various application.
- Minimized RNA damage: RNA damage is minimized by avoiding precipitation and use of organic solvents.
- High binding capacity: Uses silica-based RNA binding column with a high binding capacity of up to 120 µg.

Experimental Procedures

Before You Begin

Before proceeding, please check the following:

1. Before harvesting bacterial cultures, read “Optimal Culture Conditions” on page 6.

2. Add 10 µl of β-mercaptoethanol per 1 ml of RB Buffer.

3. Completely dissolve Proteinase K powder in 1,250 µl of nuclease-free water before use. Dissolved Proteinase K should be stored at 4°C.

4. Prepare TE Buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0) containing 20 mg/ml lysozyme.

5. Prepare 50 mg of acid-washed glass beads (150-600 µm) per sample from gram-positive bacterial cells.

Optimal Culture Conditions

RNA yield is affected by the age of the bacteria cells. Therefore, we recommend the use of fresh cultures. Bacteria cells should be harvested in exponential phase. In this phase, cells do not lose nutrients and have the highest RNA level due to their high metabolic activity. In addition, when bacterial cells reach stationary phase, the cell wall becomes much harder to penetrate, which may reduce the RNA yield. The number of bacterial cells is calculated by measuring OD600 readings. OD600 represent physiological status of cells. Therefore, we recommend using bacterial cells in the proper OD600 range of 0.2 to 0.4. In this range, bacterial cells are usually in exponential phase. In the case of *E. coli*, OD600 of 0.2 shows 1.6 x 10^8 cells per ml typically.

*** Note:** This does not apply to all cases. Therefore, you should modify the conditions.

RNA Extraction from Gram-Negative Bacterial Cells

1. Calculate the volume of bacterial culture (1 volume) and apply it to a clean 2 ml tube (not provided).
   * **Note:** Refer to the "Optimal Culture Conditions" on page 6 and determine the correct amount of cells.

2. Add 0.5 volume of RS Buffer to a bacterial culture from step 1. Mix briefly by vortexing for 5 seconds and incubate at room temperature for 5 minutes.
   * **Note:** If calculated volume of bacterial culture is 500 µl, add 250 µl of RS Buffer.

3. Centrifuge at 7,500 rpm for 10 minutes to pellet cells. Discard the supernatant carefully†.
   * **Note:** Remove residual supernatant by dabbing the tube onto a paper towel.
   † Do not remove supernatant with a pipette which may lead to loss of pellet.

4. Add 20 µl of Proteinase K to the 100 µl of TE Buffer containing lysozyme and transfer it to the tube with cell pellet from step 3.

5. Resuspend the cell pellet by pipetting and vortexing for 10 seconds. Incubate at room temperature for 10 minutes.

6. Add 700 µl of RB Buffer and mix briefly by vortexing for 10 seconds.

7. Add 500 µl of absolute ethanol† (not provided) to the lysate and mix immediately by pipetting.
   * **Note:** Do not centrifuge.
   † When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This does not affect the procedure.

8. Transfer up to sample to 700 µl of sample to the *AccuPrep*® Binding Column-III fit in a 2 ml collection tube. Close the lid gently and centrifuge at ≥14,000 rpm for 20 seconds. Discard the flow through†. Reuse the collection tube in step 9.
   * **Note:** If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same *AccuPrep*® Binding Column-III and discard the flow through.
   † Discard the flow through after each centrifugation.

9. Wash the *AccuPrep*® Binding Column-III by adding 700 µl of RWA1 Buffer.

10. Close the lid gently and centrifuge at 14,000 rpm for 20 seconds. Discard the flow through.

Reuse the collection tube in step 11.

*** Note:** After centrifugation, carefully remove the *AccuPrep*® Binding Column-III from the collection tube so that the column does not contact the flow through.

11. Wash the *AccuPrep*® Binding Column-III by adding 500 µl of RWA2 Buffer.

12. Close the lid gently and centrifuge at 14,000 rpm for 20 seconds. Discard the flow through. Reuse the collection tube in step 13.

13. Wash the *AccuPrep*® Binding Column-III by adding 500 µl of RWA2 Buffer.

14. Close the lid gently and centrifuge at 14,000 rpm for 2 minutes. Discard the flow through. Reuse the collection tube in step 15. The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution.

*** Note:** Residual ethanol may interfere with downstream reactions. After centrifugation, carefully remove the *AccuPrep*® Binding Column-III from the collection tube so that the column does not contact the flow through.

15. Centrifuge once more at 14,000 rpm for 1 minute to remove residual ethanol completely and check whether there is no droplet clinging to the bottom of the binding column.

16. Place the *AccuPrep*® Binding Column-III in a clean 1.5 ml Tube (supplied with a kit). Add 50-200 µl of ER Buffer or RNase-free water to elute RNA.

17. Incubate at room temperature for 1 minute to be absorbed the ER Buffer completely into the glass fiber of the binding column.

18. Close the lid gently and centrifuge at 10,000 rpm for 1 minute.

19. To recover more RNA (>30 µg), repeat once more elution step using the eluate from step 18.

20. Close the lid gently and centrifuge at 10,000 rpm for 1 minute.

RNA Extraction from Gram-Positive Bacterial Cells

1. Weigh 50 mg of acid-washed glass beads (150-600 µm) in a clean 2 ml tube (not provided) for use in step 8.

2. Calculate the volume of bacterial culture (1 volume) and apply it to a clean 2 ml tube (not provided).
   * **Note:** Refer to the “Optimal Culture Conditions” on page 6 and determine the correct amount of cells.

3. Add 0.5 volume of RS Buffer to a bacterial culture from step 2. Mix briefly by vortexing for 5 seconds and incubate at room temperature for 5 minutes.
   * **Note:** If calculated volume of bacterial culture is 500 µl, add 250 µl of RS Buffer.

4. Centrifuge at 7,500 rpm for 10 minutes to pellet cells. Discard the supernatant carefully†.
   * **Note:** Remove residual supernatant by dabbing the tube onto a paper towel.
   † Do not remove supernatant with a pipette which may lead to loss of pellet.

5. Add 20 µl of Proteinase K to the 100 µl of TE Buffer containing lysozyme and transfer it to the tube with cell pellet from step 4.

6. Resuspend the cell pellet by pipetting and vortexing for 10 seconds. Incubate at room temperature for 10 minutes.

7. Add 700 µl of RB Buffer and mix briefly by vortexing for 10 seconds.

8. Transfer the suspension from step 7 to a 2 ml tube containing the acid-washed glass beads prepare in step 1.

9. Disrupt the cells using bead beater (e.g. *TissueLyser*™) for 5 minutes at 50 Hz.

10. Centrifuge at maximum speed for 10 seconds and transfer the supernatant to a new 2 ml tube (not provided).

11. Add 500 µl of absolute ethanol† (not provided) to the lysate and mix immediately by pipetting.
    * **Note:** Do not centrifuge.
    † When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This

does not affect the procedure.

12. Transfer up to sample to 700 µl of sample to the *AccuPrep*® Binding Column-III fit in a 2 ml collection tube. Close the lid gently and centrifuge at ≥14,000 rpm for 20 seconds. Discard the flow through†. Reuse the collection tube in step 13.

*** Note:** If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same *AccuPrep*® Binding Column-III and discard the flow through.

† Discard the flow through after each centrifugation.

13. Wash the *AccuPrep*® Binding Column-III by adding 700 µl of RWA1 Buffer.

14. Close the lid gently and centrifuge at 14,000 rpm for 20 seconds. Discard the flow through. Reuse the collection tube in step 15.

*** Note:** After centrifugation, carefully remove the *AccuPrep*® Binding Column-III from the collection tube so that the column does not contact the flow through.

15. Wash the *AccuPrep*® Binding Column-III by adding 500 µl of RWA2 Buffer.

16. Close the lid gently and centrifuge at 14,000 rpm for 20 seconds. Discard the flow through. Reuse the collection tube in step 17.

17. Wash the *AccuPrep*® Binding Column-III by adding 500 µl of RWA2 Buffer.

18. Close the lid gently and centrifuge at 14,000 rpm for 2 minutes. Discard the flow through. Reuse the collection tube in step 19. The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution.

*** Note:** Residual ethanol may interfere with downstream reactions. After centrifugation, carefully remove the *AccuPrep*® Binding Column-III from the collection tube so that the column does not contact the flow through.

19. Centrifuge once more at 14,000 rpm for 1 minute to remove residual ethanol completely and check whether there is no droplet clinging to the bottom of the binding column.

20. Place the *AccuPrep*® Binding Column-III in a clean 1.5 ml Tube (supplied with a kit). Add 50-200 µl of ER Buffer or RNase-free water to elute RNA.

21. Incubate at room temperature for 1 minute to be absorbed the ER Buffer completely into the glass fiber of the binding column.

22. Close the lid gently and centrifuge at 10,000 rpm for 1 minute.

23. To recover more RNA (>30 μg), repeat once more elution step using the eluate from step 22.

24. Close the lid gently and centrifuge at 10,000 rpm for 1 minute.

RNA Clean-up

1. Adjust the sample to a volume of 100 µl with RNase-free water. Add 400 µl RB Buffer and mix well.

2. Add 300 µl of 80% ethanol to the diluted RNA and mix well by pipetting.
   * **Note:** Do not centrifuge.

3. Transfer the sample to the *AccuPrep*® Binding Column-III fit in a 2 ml collection tube. Close the lid gently and centrifuge at ≥14,000 rpm for 20 seconds. Discard the flow through†. Reuse the collection tube in step 4.
   * **Note:** After centrifugation, carefully remove the *AccuPrep*® Binding Column-III from the collection tube so that the column does not contact the flow through.
   † Discard the flow through after each centrifugation.

4. Wash the *AccuPrep*® Binding Column-III by adding 500 µl of RWA2 Buffer.

5. Close the lid gently and centrifuge at 14,000 rpm for 2 seconds. Discard the flow through. Reuse the collection tube in step 6.

6. Wash the *AccuPrep*® Binding Column-III by adding 500 µl of RWA2 Buffer.

7. Close the lid gently and centrifuge at 14,000 rpm for 2 minutes. Discard the flow through. Reuse the collection tube in step 8. The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution.

8. Centrifuge once more at 14,000 rpm for 1 minute to remove residual ethanol completely and check whether there is no droplet clinging to the bottom of the binding column.

9. Place the *AccuPrep*® Binding Column-III in a clean 1.5 ml Tube (supplied with a kit). Add 50-200 µl of ER Buffer or RNase-free water to elute RNA.

10. Incubate at room temperature for 1 minute to be absorbed the ER Buffer completely into the glass fiber of the binding column.

11. Close the lid gently and centrifuge at 10,000 rpm for 1 minute.

Troubleshooting

Problem	Comments
Low RNA yield	• **Buffers or other reagents may have been exposed to conditions that reduce their effectiveness.** Store the reagents at room temperature (15-25°C) at all times upon arrival and keep all reagent bottles tightly closed after use to preserve pH and stability and avoid contamination.
	• **Excess amount of starting sample may be used for RNA extraction.** Appropriate amount of starting sample should be used for efficient RNA extraction.
	• **Elution may have been incomplete.** Please expand the incubation time up to 3 minutes at elution step.
	• **Insufficient shaking or vortexing during lysis step may lead to low RNA yield.** Shake or mix thoroughly by vortexing during lysis step.
	• **Cell culture medium may have been incomplete.** Remove the cell culture medium as much as possible. Any leftover in the medium can lead to an inhibition of RNA extraction
Low RNA purity	• ***AccuPrep*® Binding Column-III may have been washed insufficiently.** Properly wash the binding column in the washing step. Remaining ethanol can decrease the RNA purity
Degraded RNA	• **There may have been RNase contamination.** Use a heat gun or blow dryer in a clean bench to prevent the RNase contamination in the air. Use RNase-free pipette tips and change the gloves frequently.
	• **The sample may have been stored inappropriately.** Cultured cell samples and lysed samples with RB Buffer should be stored at -80°C.

	• **Repeated freezing and thawing may degrade RNA.** Avoid repeated freezing and thawing.
Sample floating upon loading in an agarose gel	• **Sample may contain ethanol.** Floating is caused by remaining ethanol. Dry the column completely by centrifugation and make sure that no droplet is hanging from tip of the column. Remaining ethanol may also interrupt the enzymatic reaction.

Appendix A

Long term storage of RNA in formamide

1. Dissolve RNA pellet in deionized formamide.

2. Add NaCl to the final concentration of 0.2 M followed by 4 volumes of ethanol to precipitate RNA from formamide.

3. Incubate at room temperature for 10 minutes.

4. Centrifuge at 12,000 rpm for 5 minutes at room temperature.

Appendix B

Measurement of absorbance of RNA samples

The A_{260}/A_{280} ratio is a commonly used criterion for nucleic acid purity. Values for pure RNA are usually >1.8. However, the absorbance of nucleic acids at these wavelengths is dependent upon the ionic strength and pH of the medium. The change in the A_{260}/A_{280} ratio is primarily due to the decrease in the absorbance at 280 nm when the ionic strength or pH is increased. We recommend that diluting the RNA with a TE buffer for spectrophotometric assays.

1. Measure the volume of the total RNA sample.

2. Transfer 1 µl of total RNA sample to a 1.5 ml tube.

3. Add 999 µl of TE (pH 8.0) buffer and mix by pipetting.

4. Measure A_{260} and A_{280} with TE (pH 8.0) buffer as a reference blank.

5. Calculate RNA yield as follows:
   1 A_{260} unit of RNA = 40 µg/µl
   Total A_{260} = (A_{260} of diluted sample) x (dilution factor)
   Concentration (µg/ml) = (total A_{260}) x (40 µg/µl)
   Yield (µg) = (total sample volume) x (concentration)

6. Calculate the A_{260}/A_{280} ratio. Pure RNA exhibits a ratio between 1.9-2.0.

References

Coombs, N.J., Gough, A.C., and Primrose J.N. (1999) Optimisation of DNA and RNA extraction from archival formalin-fixed tissue. Nucleic Acids Research, 27(16), e12.

Reno, C., Marchuk, L., Sciore, P., Frank, C.B., and Hart, D.A. (1997) Rapid isolation of total RNA from small samples of hypocellular, dense connective tissues. Biotechniques, 22(6), 1082-1086.

Ordering Information

Description		Cat. No
AccuPrep® Bacterial RNA Extraction Kit	50 reactions	K-3143
	100 reactions	K-3142

Related Products

Description	Cat. No
Proteinase K Powder	KB-0111

Explanation of Symbols

LOT Batch Code	Consult Instructions For Use	RUO Research Use Only	Caution
Biological Risks	Contains Sufficient for <n> tests	Temperature Limitation	Manufacturer
REF Catalog Number	Do not Re-use	Use-by Date	

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