USER'S GUIDE

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AccuPrep®
Bacterial RNA Extraction Kit

REF Cat.no.:K-3142, K-3143

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AccuPrep® Bacterial RNA Extraction Kit

Kit for the extraction of RNA from bacterial cultures using spin column

User's Guide

Version No.: 0.0 (2018-08)

Please read all the information in booklet before using the unit

Bioneer Corporation
8-11, Munpyeongseo-ro, Daedeok-gu, Daejeon
34302, Republic of Korea
Tel: +82-42-930-8777
Fax: +82-42-930-8688
Email: sales@bioneer.co.kr
www.bioneer.co.kr

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.

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Contents

1. Description

AccuPrep® Bacterial RNA Extraction Kit can quickly and conveniently extract RNA from bacteria cultures using spin column. In the presence of chaotropic salt, RNA is bound to glass fibers fixed in a column. Proteins and other contaminants are removed through washing steps, and the RNA is isolated and eluted in the final elution step. The process does not require the use of organic solvents or ethanol precipitation steps, and is thus ideal for the safe and convenient extraction.

Advantages

- RNA can be prepared promptly and conveniently.
- Other cellular components besides nucleic acids, especially protein, nucleases, and other contaminants, are completely eliminated, resulting in improved efficiency and reproducibility of RT reaction.
- Damage and low yield of RNA are minimized because all steps are done without organic solvent and ethanol precipitation process.
- The extracted RNA can be used in a variety of applications.

2. Kit components

This kit is available for 50 rxn & 100 rxn preparations maintain performance for at least two years under standard storage conditions.

AccuPrep® Bacterial RNA Extraction Kit (K-3143, K-3142)

Reagents	K-3143 (50 rxn)	K-3142 (100 rxn)
RS Buffer	40 mL	80 mL
RS Buffer is stable for 2 years when stored at room temperature.		
RB Buffer	30 mL	60 mL
RB Buffer is stable for 2 years when stored at room temperature. *** NOTE**: Do not add Proteinase K directly to RB Buffer.		
RWA1 Buffer	40 mL	80 mL
RWA1 Buffer is stable for 2 years when stored closed at room temperature.		
RWA2 Buffer	70 mL	70 mL (x2)
RWA2 Buffer is stable for 2 years when stored closed at room temperature.		
ER Buffer	10 mL	20 mL
Store at room temperature.		
Proteinase K (lyophilized)	25 mg	25 mg (x2)
One vial includes 25 mg lyophilized Proteinase K. Dissolve one vial in 1,250 µl of nuclease-free water. Dissolved Proteinase K is stable when stored at 4℃ Storage at -20℃ is recommended to prolong the activity, but repeated freezing and thawing should be avoided.		

Columns and tubes	50 rxn	100 rxn
AccuPrep® Binding column-Ⅲ	50 ea	100 ea
1.5 ml tubes for elution	50 ea	50 ea (x2)

Additional required materials

- Absolute ethanol (96~100%)
- Lysozyme
- Glass bead (for gram-positive bacteria)
- Bead beater (for gram-positive bacteria)
- 1.5ml or 2 ml tubes (for preparation of lysis)
- Standard table-top microcentrifuge capable of a 13,000 x g centrifugal force (with rotor for 2 ml tubes)
- Incubator or thermal block
- Vortex mixer
- Sterilized filter pipette tip

3. 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 are not nutrient depleted, and RNA levels are highest due to 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 bacteria cells is calculated by measuring OD readings. OD values represent status of cells such as bacterial species and physiology. Furthermore, different species have different OD values. Therefore, we

recommend using bacteria cells which are indicated proper OD values between 0.2 and 0.4. In this range, bacteria cells are usually in exponential phase. Samples with OD values above 0.4 should be diluted so that the OD values fall within this range. In the case of *E.coli*, OD value of 0.2 shows 1.6×10^8 cells per ml typically.
(Note: we recommend these conditions, however, it is not correct for the all cases. Therefore, user should modify proper conditions.)

4. Experimental protocol

A. RNA extraction from gram-negative bacteria

(This protocol can be used to RNA extraction from gram-positive bacteria, but RNA yield lead to be low.)

Before you begin

- ✓ Before harvesting bacteria, read "3. Optimal culture conditions"
- ✓ Add 10 µl β-mercaptoethanol per 1 ml RB Buffer.
- ✓ Add 1,250 µl of nuclease-free water per 25 mg proteinase K.
- ✓ RB Buffer contains irritant chaotropic salt. Take appropriate laboratory safety precaution, and wear gloves when handling.

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

2) Calculate the volume of bacteria culture (1 volume).

(See above "3. Optimal culture conditions" and determining the correct amount of cells)

3) Add 0.5 volume of RS Buffer into a tube (not supplied).

(If calculated volume of bacteria culture is 500 µl, add 250 µl of RS Buffer)

4) Add 1 volume of bacteria culture to the tube and mix by vortex mixer for 5 s. Incubate for 5 min at room temperature (15 - 20 ℃).

5) Centrifuge at 7,500 rpm for 10 min.

6) Discard the supernatant from the tube.

(Remove residual supernatant by dabbing the tube onto a paper towel.)

(Note: Do not remove supernatant using pipetting which may lead to loss of pellet.)

7) Add 20 µl of proteinase K to the 100 µl of TE buffer containing lysozyme, and add the mixture to the tube.

8) Resuspend the pellet by pipetting and mix by vortex mixer for 10 s. Incubate for 10 min at room temperature (15 - 20 ℃).

9) Add 700 µl of RB Buffer to the tube and mix by vortex mixer for 10 s.

10) Add 500 µl of absolute ethanol and mix immediately by using pipette. Do not centrifuge.
(Note: When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This does not affect the procedure.)

11) Transfer the sample to the *AccuPrep*® Binding Column-Ⅲ in a 2 ml collection tube, close the lid and centrifuge at ≥14,000 rpm for 20 s.
(Note: The sample volume may exceeds maximum volume of *AccuPrep*® Binding Column-Ⅲ. Therefore, centrifuge successive aliquots in the same binding column. Discard the flow-through after each centrifugation.)

12) Discard the flow-through from the collection tube and reuse the collection tube.

13) Add 700 µl of RWA1 Buffer without wetting the rim, close the tube, and centrifuge at 14,000 rpm for 20 s.
(Note: After centrifugation, carefully remove the *AccuPrep*® Binding Column-Ⅲ from the collection tube so that the column does not contact the flow-through. If the rim of column is wet, RNA yield and purity will be less than expected results.)

14) Discard the solution from the collection tube and reuse the collection tube.

15) Add 500 µl of RWA2 Buffer without wetting the rim, close the tube, and centrifuge at 14,000 rpm for 20 s.

16) Discard the solution from the collection tube and reuse the collection tube.

17) Add 500 µl of RWA2 Buffer without wetting the rim, close the tube, and centrifuge at 14,000 rpm for 2 min.
The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution.

18) Discard the solution from the collection tube and reuse the collection tube.

19) Centrifuge once more at 14,000 rpm for 1 min to completely remove ethanol, and check that there aren't droplets clinging to the bottom of the *AccuPrep*® Binding Column-Ⅲ tube.

20) Transfer the *AccuPrep*® Binding Column-Ⅲ to a new 1.5 ml tube for elution, add 50 - 200 µl of ER Buffer onto *AccuPrep*® Binding Column-Ⅲ, and wait for at least 1 min at RT (15 - 25℃).

21) Centrifuge at 10,000 rpm for 1 min to elute.

If the expected RNA yield is >30 µg, repeat step 14 &15 using another 50 – 100 µl ER Buffer (if high RNA concentration is required). Reuse the collection tube from step 15.

(If using the eluate step, the RNA yield will be less than that obtained using a second volume of ER Buffer, but the final RNA concentration will be higher.)

B. RNA extraction from gram-positive bacteria

Before you begin

- ✓ Before harvesting bacteria, read "3. Optimal culture conditions"
- ✓ Add 10 µl β-mercaptoethanol per 1 ml RB Buffer.
- ✓ Add 1,250 µl of nuclease-free water per 25 mg proteinase K.
- ✓ RB Buffer contains irritant chaotropic salt. Take appropriate laboratory safety precaution, and wear gloves when handling.
- ✓ Prepare 50 mg acid-washed glass beads (150 - 600 µm) per sample.

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

2) Weigh 50 mg acid-washed glass beads (150 - 600 µm) in a 2 ml tube (not supplied), for use in step 11.

3) Calculate the volume of bacteria culture (1 volume).

(See above "3. Optimal culture conditions" and determining the correct amount of cells)

4) Add 0.5 volume of RS Buffer into a tube (not supplied).

(If calculated volume of bacteria culture is 500 µl, add 250 µl of RS Buffer)

5) Add 1 volume of bacteria culture to the tube and mix by vortex mixer for 5 s. Incubate for 5 min at room temperature (15 - 20 ℃).

6) Centrifuge at 7,500 rpm for 10 min.

7) Discard the supernatant from the tube.

(Remove residual supernatant by dabbing the tube onto a paper towel.)

(Note: Do not remove supernatant using pipetting which may lead to loss of pellet.)

8) Add 20 µl of proteinase K to the 100 µl of TE buffer containing lysozyme, and add the mixture to the tube.

9) Resuspend the pellet by pipetting and mix by vortex mixer for 10 s. Incubate from 10 min at room temperature (15 - 20 ℃).

10) Add 700 µl of RB Buffer to the tube and mix by vortex mixer for 10 s.

11) Transfer the suspension into a 2 ml tube containing the acid-washed glass beads prepared in step 2. Disrupt the cells using bead beater (e.g. TissueLyser™) for 5 min at 50 Hz.

12) Centrifuge for 10 s at maximum speed. Transfer the supernatant into a new tube (not supplied).

13) Add 500 µl of absolute ethanol and mix immediately by using pipette. Do not centrifuge.
(Note: When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This does not affect the procedure.)

14) Transfer the sample to the *AccuPrep*® Binding Column-Ⅲ in a 2 ml collection tube, close the lid and centrifuge at ≥14,000 rpm for 20 s.
(Note: The sample volume may exceed maximum volume of *AccuPrep*® Binding Column-Ⅲ. Therefore, centrifuge successive aliquots in the same binding column. Discard the flow-through after each centrifugation.)

15) Discard the flow-through from the collection tube and reuse the collection tube.

16) Add 700 µl of RWA1 Buffer without wetting the rim, close the tube, and centrifuge at 14,000 rpm for 20 s.
(Note: After centrifugation, carefully remove the *AccuPrep*® Binding Column-Ⅲ from the collection tube so that the column does not contact the flow-through. If the rim of column is wet, RNA yield and purity will be less than expected results.)

17) Discard the solution from the collection tube and reuse the collection tube.

18) Add 500 µl of RWA2 Buffer without wetting the rim, close the tube, and centrifuge at 14,000rpm for 20 s.

19) Discard the solution from the collection tube and reuse the collection tube.

20) Add 500 µl of RWA2 Buffer without wetting the rim, close the tube, and centrifuge at 14,000rpm for 2 min.

The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution.

21) Discard the solution from the collection tube and reuse the collection tube.

22) Centrifuge once more at 14,000 rpm for 1 min to completely remove ethanol, and check that there is no droplet clinging to the bottom of the *AccuPrep*® Binding Column-Ⅲ tube.

23) Transfer the *AccuPrep*® Binding Column-Ⅲ to a new 1.5 ml tube for elution, add 50 - 200 µl of ER Buffer onto *AccuPrep*® Binding Column-Ⅲ, and wait for at least 1 min at RT (15 - 25℃).

24) Centrifuge at 10,000 rpm for 1 min to elute.

If the expected RNA yield is >30 µg, repeat step 14 &15 using another 50–100 µl ER Buffer (if high RNA concentration is required). Reuse the collection tube from step 15.

(If using the eluate step, the RNA yield will be less than that obtained using a second volume of ER Buffer, but the final RNA concentration will be higher.)

C. 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 ethanol (80 %) to the diluted RNA, and mix immediately by using pipette. Do not centrifuge.

3) Transfer the sample to the *AccuPrep*® Binding Column-Ⅲ in a 2 ml collection tube, close the lid and centrifuge at ≥14,000 rpm for 20 s.

4) Discard the flow-through from the collection tube and reuse the collection tube.

5) Add 500 µl of RWA2 Buffer without wetting the rim, close the tube, and centrifuge at 14,000rpm for 20 s.

6) Discard the solution from the collection tube and reuse the collection tube.

7) Add 500 µl of RWA2 Buffer without wetting the rim, close the tube, and centrifuge at 14,000rpm for 2 min.

The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution.

8) Discard the solution from the collection tube and use a new collection tube.

9) Centrifuge once more at 14,000 rpm for 1 min to completely remove ethanol, and check that there is no droplet clinging to the bottom of the *AccuPrep*® Binding Column-Ⅲ tube.

10) Transfer the *AccuPrep*® Binding Column-Ⅲ to a new 1.5 ml tube for elution, add 50 - 200 µl of ER Buffer onto *AccuPrep*® Binding Column-Ⅲ, and wait for at least 1 min at RT (15 - 25℃).

11) Centrifuge at 10,000 rpm for 1 min to elute.

5. Appendix

A. Troubleshooting guide

If you have problems during RNA extraction, please use the Troubleshooting Guide.

This troubleshooting guide will help you to solve problem that may arise during RNA extraction. For other technical assistance or more information, please contact our technical assistance team.

	Comments and suggestions
Low yield of RNA	Buffers or other reagents may have been exposed to external factors that may have reduced its quality. Please make sure that reagents are stored at room temperature at all times upon arrival and that all reagent bottles are closed tightly, in order to preserve pH and stability, and to avoid contamination.
	Excess amount of starting sample was used to extract DNA. Appropriate amount of starting sample should be used for efficient extraction of RNA.
	Elution may have been incomplete. Please extend incubation time up to 3 minutes at elution step to improve the RNA.
	Insufficient shaking or vortexing during lysis step may lead to low RNA yield than expected. Shake or mix with a vortex mixer sufficiently during incubation step.
	Cell culture medium may have been incomplete. The best approach is to remove the medium as much as possible. Any leftover in the medium can lead to the inhibition of RNA extraction.
Low $A_{260/280}$ ratio	Spin column may have been washed insufficiently. You must properly wash the spin column in the washing step. Remaining ethanol can decrease the purity of RNA.
Degraded RNA	RNase contamination can be degraded RNA. Use a heat gun or a blow dryer in a clean bench to prevent the contamination of RNase in the air. Use RNase-free pipette tips and change the gloves frequently.
	Cultured cell samples that have been stored at -80℃ or lysis the samples with RB Buffer and then store at -80℃.
	Frequent freezing and thawing may result in lower RNA yield than expected. Avoid repeated freezing and thawing.
Flotation of extracted RNA when loaded on an agarose gel	Floating of RNA on an agarose gel is caused by the remaining ethanol in the eluted RNA. Ensure that the ethanol removing step in the protocol is properly performed. Remaining ethanol may also interrupt the enzymatic reaction.

B. Long term storage of RNA in formamide

1) Dissolve RNA pellet in deionized formamide.
2) To precipitate RNA from formamide, add NaCl to final concentration of 0.2 M followed by 4 volumes of ethanol.
3) Incubate at room temperature for 10 min.
4) Centrifuge at 12,000 rpm for 5 min at room temperature.

C. Measuring 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 RNA be diluted with TE buffer for spectrophotometric assays.

1) Measure the total RNA sample volume.
2) Transfer 1 μl of your total RNA sample to a 1.5 ml tube.
3) Add 999 μl of TE (pH8.0) buffer to the 1.5 ml tube and mix by pipetting.
4) Measure A_{260} and A_{280} using TE (pH8.0) buffer as a reference blank.
5) Calculate RNA yield as follows:

   $1A_{260}$ unit of RNA = 40 μg/μl

   Total A_{260} = (A_{260} of dilute 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.

   Example) The volume of RNA sample was 0.5 ml. One 1μl sample aliquot was diluted to 1.0 ml in TE buffer (pH8.0), and the spectrophotometric readings were taken: A_{260} = 0.231, A_{280} = 0.115

   Caculations) Total A_{260} = (0.131) x (1000) = 131

   Concentration = (131) x (40) = 5,240 μg/ml

   RNA yield = (0.5 ml) x (5,240 μg/ml) = 2,620 μg

   Purity = 0.131 / 0.065 = 2.01

6. References

1) N. J. Coombs.*et al.* (1999) *Nucleic Acids Res.*, Vol **27**, No.16
2) C. Reno.*et al.* (1997) *Biotechniques* , Vol **22**, No. 6
3) Michael J.Bonham.*et al.* (1996) *Biotechniques*, Vol **20**, No. 5

Bioneer Worldwide

Bioneer Corporation

Address	8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon, 34302, Republic of Korea
Tel	+82-42-930-8777 (Korea: 1588-9788)
Fax	+82-42-930-8688
E-mail	sales@bioneer.com
Web	www.bioneer.com

Bioneer Inc.

Address	155 Filbert St. Suite 216 Oakland, CA 94607, USA
Tel	+1-877-264-4300 (Toll-free)
Fax	+1-510-865-0350
E-mail	orderusa@bioneer.com
Web	us.bioneer.com

Bioneer R&D Center

Address	Korea Bio Park BLDG #B-702, 700 Daewangpangyo-ro, Bundang-gu, Seongnam-si Gyeonggi-do, 13488, Republic of Korea
Tel	+82-31-628-0500
Fax	+82-31-628-0555
E-mail	sales@bioneer.co.kr
Web	www.bioneer.co.kr