USER GUIDE

AccuPrep®
Universal RNA Extraction Kit

Cat. No. K-3140
K-3141

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AccuPrep® Universal RNA Extraction Kit

Kit for the extraction of RNA from cultured cells, plant tissues, or animal tissues

User Guide

K-3141 Σ **50**

K-3140 Σ **100**

Version No.: 1.0 (2022-03-22)

Please read all the information in booklet before using the unit

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Intended Use

AccuPrep® Universal 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).

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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

Product Information

Components

Components	K-3140 (100 reactions)	K-3141 (50 reactions)	Storage
RB Buffer (Binding)	56 ml	28 ml	Store at room temperature (15-25°C).
RWA1 Buffer (1st Washing)	80 ml	40 ml	
RWA2 Buffer (2nd Washing)	70 ml x 2 ea	70 ml	
ER Buffer (Elution)	20 ml	10 ml	
AccuPrep® Binding Column-III	100 ea	50 ea	
1.5 ml Tube (Elution)	100 ea	50 ea	
Collection Tube (Filtration)	100 ea	50 ea	
One Page Protocol	1 ea	1 ea	

Storage

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

Specifications

AccuPrep® Universal RNA Extraction Kit		
Amount of Starting Sample	Cultured cells	10^4-10^8 cells
	Animal tissue	25-50 mg
	Plant tissue	100 mg
Typical RNA Yield	Cultured cells	15-20 µg
	Animal tissue	10-60 µg
	Plant tissue	30-60 µg
Column Binding Capacity		up to 120 µg
Column Loading Volume		800 µl
Elution Volume		30-100 µl
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® Universal RNA Extraction Kit is designed for extraction of highly purified RNA from cultured cells, plants and animal tissues within 20 minutes. The kit employs *AccuPrep*® Binding Column-III with silica membrane for nucleic acid binding in the presence of chaotropic salts. Samples are lysed and homogenized in the presence of a guanidine-thiocyanate-containing buffer, which is a highly denaturing agent and inactivates RNase to isolate RNA. After homogenization, 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 total RNA extraction from various samples such as plant tissues, animal tissues, or cultured cells.
- Convenient & Rapid: The process does not require phenol/chloroform extraction, ethanol precipitation, or other time-consuming steps.
- Efficient: Contaminants such as proteins and nucleases are completely removed.
- 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. Add 10 µl of β-mercaptoethanol per 1 ml of RB Buffer.

2. g-force can be calculated as follows: rcf = 1.12 x r x $(rpm/1,000)^2$

   * **Note:** Where 'rcf' is the relative centrifugal force (in g), 'r' is the radius of the rotor in centimeters, and rpm is the speed of the centrifuge in revolutions per minute.

Preparing Lysates from Cultured Cells

1. **(Cell harvesting)** Harvest cells according to step 1-A or 1-B.

1-A. Suspension cell culture:

Harvest cultured cells (10^4-10^6 cells) by centrifugation at 300 x *g* for 5 minutes to pellet cells. Discard the supernatant carefully without disturbing a cell pellet and go to step 2.

1-B. Monolayer cell culture:

There are two different methods to collect cells grown in monolayer culture.

a. Direct cell harvesting on the culture dishes:
   Completely discard the cell culture medium and go to step 2.
   * **Note**: You should completely remove the cell culture medium because it may inhibit the RNA extraction.

b. Cell harvesting with trypsin:
   Remove the cell culture medium and wash the cell monolayer with DPBS. Add 0.1-0.25% trypsin to the washed cell monolayer to detach the cells. After the cells detach, add cell culture medium. Transfer the cells to an RNase-free tube (not provided) and centrifuge at 300 x *g* for 5 minutes. Discard the supernatant carefully and go to step 2.

2. **(Lysis & homogenization)** Resuspend the cell pellet from step 1 in 400 µl of RB Buffer by vortexing.
   * **Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.

3. **(RNA precipitation)** Add 300 µl of 80% ethanol† 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.

4. Proceed immediately to “Purification Procedure Using Spin Columns” on page 9.

Preparing Lysates from Plant Tissue

1. **(Sample preparation)** Grind ≤100 mg of plant sample in liquid nitrogen† to a fine powder with a mortar and pestle and place it into a clean 1.5 ml tube.

   *** Note:** Do not allow the sample to thaw.

   † After grinding, liquid nitrogen should be evaporated.

2. **(Lysis & homogenization)** Add 500 µl of RB Buffer to the sample and mix thoroughly by vortexing.

3. Incubate at 60°C for 1-3 minutes. A short 1-3 minutes incubation at 60°C may help to disrupt the tissue. Centrifuge at full speed for 2 minutes and transfer the supernatant to a new 1.5 ml tube.

   *** Note: (Optional)** Centrifugation through the *AccuPrep*® Filtering Column (Cat. No. KA-1160) removes debris effectively.

4. **(RNA precipitation)** Add 0.5 volume of absolute ethanol (not provided) to the lysate and mix immediately by pipetting.

   *** Note:** Do not centrifuge.

5. Proceed immediately to “Purification Procedure Using Spin Columns” on page 9.

Preparing Lysates from Animal Tissue

1. **(Lysis & homogenization)** Grind (or homogenize) 20-30 mg of fresh or thawed animal tissue sample with a mortar and pestle (or homogenizer) and place it into a clean 1.5 ml tube.

2. Add 500 µl of RB Buffer to the sample and mix thoroughly by vortexing.

3. Centrifuge at full speed for 3 minutes and carefully transfer the supernatant to a new 1.5 ml tube.
   * **Note: (Optional)** Centrifugation through the *AccuPrep*® Filtering Column (Cat. No. KA-1160) removes debris effectively.

4. **(RNA precipitation)** Add 200 µl of absolute ethanol (not provided) to the lysate and mix immediately by pipetting.
   * **Note:** Do not centrifuge.

5. Proceed immediately to “Purification Procedure Using Spin Columns” on page 9.

Purification Procedure Using Spin Columns

1. **(RNA binding)** Transfer up 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 2.
   * **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.

2. **(1st Washing)** Wash the *AccuPrep*® Binding Column-III by adding 700 µl of RWA1 Buffer.

3. 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.

4. **(2nd Washing)** 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 20 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.
   * **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.

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. **(Elution)** 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.

12. To recover more RNA (>30 μg), repeat once more elution step using the eluate from step 11.

13. 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.

Summary of reagent volumes required in each step of RNA extraction

RNA Extraction from Cultured Cells

Step	Buffer	Volume
Cell Lysis & Biding	RB Buffer	400 µl
RNA Precipitation	80% Ethanol	300 µl
1st Washing	RWA1 Buffer	700 µl
2nd Washing (x2)	RWA2 Buffer	500 µl
Elution	ER Buffer	50-200 µl

RNA Extraction from Plant Tissue

Step	Buffer	Volume
Cell Lysis & Biding	RB Buffer	500 µl
RNA Precipitation	Absolute Ethanol	0.5 volume
1st Washing	RWA1 Buffer	700 µl
2nd Washing (x2)	RWA2 Buffer	500 µl
Elution	ER Buffer	50-200 µl

RNA Extraction from Animal Tissue

Step	Buffer	Volume
Cell Lysis & Biding	RB Buffer	500 µl
RNA Precipitation	Absolute Ethanol	200 µl
1st Washing	RWA1 Buffer	700 µl
2nd Washing (x2)	RWA2 Buffer	500 µl
Elution	ER Buffer	50-200 µl

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® Universal RNA Extraction Kit	50 reactions	K-3141
	100 reactions	K-3140

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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