USER GUIDE

AccuPrep®
Viral RNA Extraction Kit

Cat. No. K-3033

BIONEER
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AccuPrep® Viral RNA Extraction Kit

Kit for the extraction of RNA from virus

User Guide

K-3033

Σ 100

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Please read all the information in booklet before using the unit

BIONEER Corporation
Bioneer Global Center, 71, Techno-2-ro,
Yuseong-gu, Daejeon, 34013, Republic of Korea
Tel: 1588-9788
Email: sales@bioneer.co.kr
www.bioneer.com

Intended Use

AccuPrep® Viral 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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Copyright

Notice

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Contents

Product Information

Components

This kit contains adequate reagents for 100 reactions.

Components	Amount	Storage
Proteinase K powder, lyophilized	25 mg	Refer to the “Storage” below.
Poly (A), lyophilized	2 mg	
VB Buffer (Binding)	50 ml	Store at room temperature (15-25°C).
VW1 Buffer (1st Washing)	40 ml	
RWA2 Buffer (2nd Washing)	70 ml	
ER Buffer (Elution)	20 ml	
BST Solution	40 ml	
AccuPrep® Binding Column-I	100 ea	
Collection Tube (Filtration)	100 ea	
1.5 ml Tube (Elution)	100 ea	
One Page Protocol	1 ea	

Storage

The kit will maintain performance for at least three 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 dissolved in 1,250 µl of nuclease-free water and Poly (A) should be dissolved in 500 µl of ER Buffer, respectively. For short term storage, dissolved Proteinase K and Poly (A) should be stored at 4°C. For long term storage, it is recommended to aliquot these enzymes into separate tubes and store at -20°C.

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

Specifications

Sample Type	Amount of Starting Sample	Typical RNA Yield
Serum	200 µl	>90% recovery
Plasma		
Cell-free body fluid		
Swab	1 ea	

*** 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 VB Buffer and VW1 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® Viral RNA Extraction Kit is designed for extraction of highly purified viral RNA from serum, plasma, or other cell-free body fluids. The kit employs *AccuPrep*® Binding Column-I with glass fiber filter for nucleic acid binding in the presence of chaotropic salts. The RNA binds to the silica of glass fiber, while proteins and other contaminants are eliminated through a series of short wash-and-spin steps using ethanol. Finally viral RNA is eluted by low salt solution. 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, pathogen detection and cDNA synthesis. We recommend DNase treatment for only RNA quantitation.

Features & Benefits

- Comprehensive: High quality and yield of viral RNA extraction from various samples such as serum, urine, or swab.
- 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.

Experimental Procedures

Before You Begin

Before proceeding, please check the following:

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

2. Dissolve Poly(A) in 500 µl of ER Buffer and mix gently by vortexing.

3. Choose one from two ways after dissolving Poly(A).

A. Add dissolved Poly(A) solution to VB Buffer and mix thoroughly.

***Note:** In this case, Poly(A) can be degraded by RNase under the external environment. So, it is recommended to use and store it on a clean bench.

B. Add 5 µl of dissolved Poly(A) per 300 µl of VB Buffer. Divide dissolved Poly(A) into conveniently sized aliquots and store it at -20°C.

*** Note:** Do not freeze-thaw the aliquots more than 3 times.

4. Add indicated volume of absolute ethanol (not provided) to VW1 Buffer before use (see bottle label).

5. Pre-heat ER Buffer at 60°C before use. This process is essential for maximal recovery in filter-based extraction.

Viral RNA Extraction

1. Apply 200 µl of serum, plasma, or urine sample to a 1.5 ml or 2 ml tube (not provided).
   * **Note:** For swab sample, add PBS buffer (not provided) to the sample and mix by vortexing. Use only supernatant.

2. Add 10 µl of Proteinase K to the sample from step 1.

3. Add 300 µl of VB Buffer† and mix briefly by vortexing for 10 seconds.
   * **Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.
   † Refer to "Before You Begin" on page 5.

4. Incubate at 56-60°C for 10 minutes.

5. Add 300 µl of isopropanol (not provided) and mix briefly by vortexing for 10 seconds. Briefly spin down to collect lysate clinging under the lid.

6. Add 100 µl of BST Solution to the *AccuPrep*® Binding Column-I fit in a Collection Tube.

7. Close the lid gently and centrifuge at 13,000 rpm for 30 seconds. Discard the flow through. Reuse the collection tube in step 8.

8. Apply the cleared lysate from step 5 to the *AccuPrep*® Binding Column-I fit in a Collection Tube from step 6.

9. Close the lid gently and centrifuge at 13,000 rpm for 1 minute. Discard the flow through. Reuse the collection tube in step 10.

10. Wash the *AccuPrep*® Binding Column-I by adding 500 µl of VW1 Buffer.

11. Close the lid gently and centrifuge at 13,000 rpm for 1 minute. Discard the flow through. Reuse the collection tube in step 12.
    * **Note:** After centrifugation, carefully remove the *AccuPrep*® Binding Column-I from the Collection Tube so that the column does not contact the flow through.

12. Wash the *AccuPrep*® Binding Column-I by adding 600 µl of RWA2 Buffer.

13. Close the lid gently and centrifuge at 13,000 rpm for 1 minute. Discard the flow through. Reuse the collection tube in step 14.

14. 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.

15. Place the *AccuPrep*® Binding Column-I in a clean 1.5 ml Tube (supplied with a kit). Add 100 µl of ER Buffer or RNase-free water to elute RNA and let stand for 1 minute. Close the lid gently and centrifuge at 13,000 rpm for 1 minute.

    ***Note:** A smaller volume will result in a more concentrated solution, but total yield may be reduced. We recommend letting stand for about 5 minutes to increase RNA yield. Pre-warmed ER Buffer will also improve efficiency of elution (see “Before You Begin” on page 5).

16. To recover more RNA, repeat once more elution step using the eluate from step 15.

17. Close the lid gently and centrifuge at 13,000 rpm for 1 minute.

Viral DNA Extraction

1. Apply 200 µl of serum, plasma, or urine sample to a 1.5 ml or 2 ml tube (not provided).
   * **Note:** For swab sample, add PBS buffer (not provided) to the sample and mix by vortexing. Use only supernatant.

2. Add 10 µl of Proteinase K to the sample from step 1.

3. Add 200 µl of VB Buffer† and mix briefly by vortexing for 10 seconds.
   * **Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.
   † Refer to “Before You Begin” on page 5.

4. Incubate at 56-60°C for 10 minutes.

5. Add 400 µl of isopropanol (not provided) and mix briefly by vortexing for 10 seconds. Briefly spin down to collect lysate clinging under the lid.

6. Go to step 6 of “Viral RNA Extraction” on page 6 and continue the instructions accordingly.

Troubleshooting

Problem	Comments
Low RNA yield or purity	• **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.
	• **Poly(A) may not have been completely mixed with VB Buffer.** When adding all dissolved Poly(A) to the VB Buffer, mix completely by vortexing.
	• **VB Buffer containing dissolved Poly(A) may have been contaminated.** To prevent RNase contamination, we recommend to use and store VB Buffer on a clean bench. Use Poly(A) separately from VB Buffer (Refer to the no.3 of “Before You Begin” on page 5).
	• **Ethanol may not have been added to VW1 Buffer.** Add absolute ethanol (not provided) to the VW1 Buffer (see “Before You Begin” on page 5) and mix well. Mark VW1 Buffer bottle label to indicate whether ethanol has been added or not.
	• **Reagents and samples may not have been completely mixed.** Always mix the sample tube well after adding each reagent.
High absorbance at 260 nm	• VB Buffer in the kit needs to be added Poly(A) as RNA carrier for enhancing efficiency of RNA extraction. Because Poly(A) has UV absorbance and it is more abundant than viral RNA, extracted RNA can show high absorbance at 260 nm. For quantification of extracted viral RNA, we recommend amplification.

Ordering Information

Description		Cat. No
AccuPrep® Viral RNA Extraction Kit	100 reactions	K-3033

Related Products

Description	Cat. No
Proteinase K Powder	KB-0111
Poly (A)	KB-0121
Phosphate Buffered Saline (PBS)	C-9024

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	

BIONEER Corporation - HQ

Address 8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon, 34302, Republic of Korea
E-mail sales@bioneer.co.kr
Web www.bioneer.com

BIONEER Global Center

Address 71, Techno 2-ro, Yuseong-gu, Daejeon, 34013, Republic of Korea
E-mail sales@bioneer.co.kr
Web www.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
E-mail sales@bioneer.co.kr
Web www.bioneer.com

BIONEER Inc. - USA Branch

Address 155 Filbert St. Suite 216 Oakland, CA 94607, USA
E-mail order.usa@bioneer.com
Web us.bioneer.com

BIONEER Corp. - European Branch

Address Ludwig-Erhard-Strasse 30-34, 65760 Eschborn, Germany
E-mail euinfo@bioneer.com
Web www.bioneer.com

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