ISO 9001
Certified

User's Guide

Global Genomics Partner

AccuPrep® Viral RNA Extraction Kit

Cat. No.: K-3033

BIONEER
bioneer corporation

Safety Warnings and Precautions

This kit is for research use only, and should not be used for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Always wear gloves when treating irritants or harmful reagents.

Warranty and Liability

All BIONEER products meet strict Quality Control standards, and are warranted to perform as described when used correctly. Problems should be reported immediately, and any liability incurred by BIONEER to the customer is limited to the replacement of the products. The customer must provide full details of the problem to BIONEER within 30 days, and return the product to BIONEER for examination.

Quality Management System ISO 9001 Certified

All aspects of our quality management system, from product development and production to quality assurance and supplier qualification, have been certified to meet world-class standards.

QC Testing

Each lot of BIONEER's product is tested in our quality control team as raw material prior to purchase. Acceptable lots are processed and tested again as finished product.
Prior to purchase, each lot of the product is tested by BIONEER's quality control team as raw materials. The acceptable lots are processed and retested as a finished product.

Trademarks

AccuPrep® is trademark of Bioneer Corporation.

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AccuPrep® Viral RNA Extraction **Kit** ***Manual***

I. Introduction

The *AccuPrep*® Viral RNA Extraction Kit can quickly and conveniently extract RNA from Serum or Plasma. 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 is not require the use of organic solvents or ethanol precipitation steps, and is thus ideal for the safe and convenient extraction.

Advantages :

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

II. Kit Components

The product has been designed to perform 100 purifications, and will maitain its performance for at least a year.

K-3033 *AccuPrep*® Viral RNA Extraction Kit

Reagents	
Poly (A), lyophilized	2 mg
One vial with 2 mg of lyophilized Poly(A). Dissolve in RNase-free water.	
VB Buffer	60 mL
Mix VB buffer thoroughly by shaking before use. VB buffer is stable for 1 year when stored at room temperature. **NOTE:** Check the adding Poly(A).	
Washing buffer 1	40 mL
W1 buffer is supplied as a concentrate. Before using for the first time, add 30ml of absolute ethanol. W1 buffer is stable for 1 year when stored closed at room temperature.	
Washing buffer 2	20 mL
W2 buffer is supplied as a concentrate. Before using for the first time, add 80ml of absolute ethanol. W2 buffer is stable for 1 year when stored closed at room temperature.	
EL Buffer	10 ml
Store at room temperature.	
Columns and tubes	
RNA-Binding column tubes	100 ea
2 mL tubes for filtration	100 ea
1.5 mL tubes for elution	100 ea

III. Required Reagents and Equipment

1. Absolute ethanol (98 ~100%)
2. Absolute isopropyl alcohol
3. Standard table-top microcentrifuge capable of a 13,000 x g centrifugal force (with rotor for 2 ml tubes)
4. Incubator or thermal block.

IV. Before You Begin

Before you proceed, check if you have done the below.

1. Did you dissolve Poly(A) in the bottle of VB buffer?
2. Did you add adequate amount of absolute ethanol to W1 buffer and W2 buffer? If not, add absolute ethanol before use.
3. Before starting a extraction process, warm the Elution buffer (EL) to 70℃

☞ The VB buffer contains irritant chaotropic salt. Take appropriate laboratory safety precaution, and wear gloves when handling.

☞ If everything is prepared, you can proceed!

V. Experimental Procedure

Extracting RNA from Serum or Plasma.

1. Add 200μl of Serum or Plasma to a 1.5ml eppendorf tube.

2. Add 400ul of Binding buffer(VB) in the tube and mix by lightly vortexing for 5 sec.
   To ensure efficient lysis, the sample should be mixed thoroughly with buffer VB to yield.

3. Incubate for 10 minutes at room temperature.

4. Add 100μl of isopropanol, lightly vortex for about 5 seconds, then spin down for 10 seconds to down the liquid clinging to the walls and lid of the tube.

5. Fit the binding column into the 2ml collection tube. Transfer the liquid into the binding column, not getting the lid wet.

6. Close lid carefully and centrifuge for 1min. at 8,000 rpm.
   If the liquid has not completely passed the column following centrifugation, then centrifuge again until the liquid completely passes through.

7. Following centrifugation, transfer the binding column to a new 2 ml collection tube.

8. Add 500μl of W1 buffer to the column, not getting the sides wet; close the lid, and centrifuge for 1min. at 8,000 rpm.

9. After centrifugation, transfer the binding column to 2 ml collection tube.

10. Add 500μl of W2 buffer, not getting the sides wet; close the lid, and centrifuge for 1 minute at 8,000 rpm.
    Residual W2 buffer in the column may cause problems in later applications.

11. Spin down once more at 13,000 rpm for 1 minute to remove ethanol completely. Make sure that there is no droplet hanging from the bottom of the binding column.
    Residual W2 buffer left in the binding column may cause problems in later applications.

12. Transfer the binding column to a 1.5 ml collection tube, add 50μl of Elution Buffer, and let stand for 1 minute to allow the buffer to permeate the column.
    We recommend letting stand for about 5 minutes to increase RNA yield.
    You can also increase yield by heating the Elution Buffer at about 60℃ before adding to the column.

13. Elute by spinning down at 8,000 rpm for 1 minute.
    The eluted RNA solution can directly be used,
    or stored at -70℃ for longer storage.

VI. Problem Solving

1. Yield or Purity of RNA is low.

1) The kit may have been stored under non-optimal conditions. → Store kit at 15-25℃ at all times upon arrival.
2) Mix VB Buffer completely after adding Poly(A).
3) Ethanol may not have been added to the Washing Buffers. → Add absolute ethanol to all Wash Buffers before using. After adding ethanol, mix the Wash Buffer well and store at 15 ~ 25℃. Always mark the Wash Buffer vial to indicate whether ethanol has been added or not.
4) Reagents and samples may not have been completely mixed. → Always mix the sample tube throughly after adding each reagent.

2. There is a low recovery of RNA following elution.

1) You may not have used the optimal reagent for RNA elution. An alkaline pH is required for optimal elution. → Next time, do not use water to elute RNA. Use the Elution Buffer included in the kit.

VII. Supplement

1. 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 10min.
4) Centrifuge at 12,000 rpm for 5 min at room temperature.

2. 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 0.5ml. One 1μl sample aliquot was diluted to 1.0 ml in TE(pH8.0) buffer, 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

VIII. 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

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