USER'S GUIDE

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AccuPrep® Dx Viral RNA Extraction Kit

REF **K-3033M**

IVD Viral RNA Extraction Kit

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AccuPrep® Dx Viral RNA Extraction Kit

Kit for the extraction of RNA from virus

User's Guide

100

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Please read all the information in booklet before using the unit

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Safety warning and Precaution

Before, during and after use of this kit as described in this User Manual, all potentially hazardous materials (i.e. materials that may have come in contact with clinical samples) including tubes, tips and materials should be processed and disposed of according to applicable and appropriate regulations of the municipality/government in which this product is being used. Please read the User Manual before using this Kit. Adhere to general clinical laboratory safety procedures during the experiment. Do not reuse reagents and take care not to mix used and fresh reagents. Wear appropriate protection when handling any irritant or harmful reagents. The use of a laboratory coat, protective gloves and safety goggles are highly recommended. Please inquire BIONEER's Customer Service Center to obtain a copy of the Material Safety Data Sheet (MSDS) for this product.

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.

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Contents

1. Intended Use

AccuPrep® Dx Viral RNA Extraction Kit is an *in vitro* diagnostic kit designed for the extraction of viral RNA from various human samples such as serum, plasma, CSF, swab and sputum. This product consists of silica-based RNA binding column, binding buffer, washing buffers elution buffer optimized for viral RNA extraction from the various sample. Furthermore, Poly (A) is included in the product for improved efficiency and preventing for RNA degradation. This kit is a nucleic acid extraction for professional use only.

2. Overview

AccuPrep® Dx Viral RNA Extraction Kit can quickly and conveniently extract viral RNA from serum, plasma, CSF, swab or sputum. 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.

Protocol is suitable for use with plasma, serum and cell-free body fluids. This kit can be used for isolation of viral RNA from broad of RNA viruses.

This product can separate comprehensive types of viral RNA from HIV, HAV, HCV, enterovirus, etc. The isolated RNA can be used for a wide range of experiments such as RT-PCR and Quantitative Real time RT-PCR, etc.

3. Kit components

※ This kit provides for 100 preparations and will maintain performance for at least two years under standard storage conditions.

Reagents		
Poly (A), lyophilized	**2 mg (KB-0122)**	**1 ea**
One vial with 2 mg of lyophilized Poly(A). Dissolve in ER Buffer.		
Proteinase K, lyophilized	**25 mg (KB-0114)**	**1 ea**
One vial with 25 mg of lyophilized Proteinase K. Dissolve in ER Buffer. 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.		
VB Buffer	**50 ml (KB-2052)**	**1 ea**
Mix VB Buffer thoroughly by shaking before use. VB Buffer is stable for 2 years when stored at room temperature.		
NOTE: Check the adding Poly (A).		
BST Solution	**40 ml (KB-1067)**	**1 ea**
Store at room temperature.		
VW1 Buffer	**40 ml (KB-3042)**	**1 ea**
VW1 Buffer is supplied as a concentrate. Before using for the first time, add 30ml of absolute ethanol. VW1 buffer is stable for 2 years when stored closed at room temperature.		
RWA2 Buffer	**70 ml (KB-4023)**	**1 ea**
RWA2 Buffer is stable for 2 years when stored closed at room temperature.		
ER Buffer	**20 ml (KB-6033)**	**1 ea**
Store at room temperature.		
Columns and tubes		
AccuPrep® Binding column-I	**50 ea (KA-1120)**	**2 pk**
Collection tubes (for filtration)	**50 ea**	**2 pk**
1.5 ml tubes (for elution)	**100 ea**	**1 pk**
Manual		
User's Guide (EN)		**1 ea**
User's Guide (KR)		**1 ea**

4. Additional required materials

- Absolute ethanol (98 – 100%)
- Absolute isopropyl alcohol
- 1.5 ㎖ tube (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

5. Storage

This kit provides for 100 preparations and will maintain performance for at least two years under standard storage conditions (Temperature: 15 – 35 ℃). 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.

6. Note

– This product is an *in vitro* diagnostic reagent and cannot be used for purposes other than diagnosis

– When handling biologically hazardous reagents and clinical specimens, wear gloves and masks.

– Perform experiments using a sterile filter pipette tip.

– Clinical samples are recommended to be frozen and stored separately from the freezer that stores the other reagents.

– Do not use the test kit if the kit or kit component is damaged or the seal is broken

– Do not use test kit beyond its expiration date

This Kit is intended to be used by a qualified clinical diagnostic staff, trained to correctly and appropriately handle and manipulate clinical specimens. A user must have basic experimental techniques for correct execution of the experiments described in this ***User's Guide.***

7. Before you begin

We recommend that several precautionary measures are taken to ensure proper results are obtained.

 All samples must be treated as infectious substances.

7.1 Appropriate bench use

Pressurized benches are divided into positive pressure and negative pressure benches. Positive pressure benches (e.g. clean benches etc.) are workspaces where filtered air flows outward, thus keeping a clean environment within the workspace. Negative pressure benches (e.g. Biosafety Cabinets, fume hoods etc.) send air from the laboratory space outside. In other words, air flows inward. This air flow prevents dangerous substances from contaminating the laboratory environment.

When handling clinical samples (especially of high pathogenicity), it is critical for the safety of the operator and other staff members that all related work (i.e. decapping, pipetting, capping of clinical samples and containers) be conducted within a negative pressure bench and preferably, a filtered bench rated for the classification of pathogens that are being handled, such as a properly-rated biosafety cabinet.

7.2 Cleaning and care of accessories

The greatest source of contamination is human-induced. To prevent unintended contamination of reagents, we recommend that the following guidelines be implemented in cleaning and maintaining the various accessories.

Discard all liquids, plastic consumables, used wipers etc. in appropriate biohazard containers according to local and national regulations.

7.3 Sample transport

All samples should be transported in a shatterproof transport container to prevent potential infection from sample leakage. Samples should be transported according to local or national guidelines regarding biohazard transportation

7.4 Sample collection and storage

- **Plasma**

  Plasma samples should be taken in a tube containing blood clotting inhibitors (EDTA and ACD). Plasma can be stored at 4°C for up to 7 days and at −70°C for up to one year. It is recommended that the frozen plasma samples be quickly dissolved in a 37°C trillion tank and stored in ice before use.

- **Serum**

  Serum samples should be collected in a tube coated with silica particle. Serum can be stored at 4°C for up to 7 days and at −70°C for up to one year. It is recommended that serum samples frozen in a 37°C tank be quickly dissolved and stored in ice before use.

- **Swab**

  Insert the swab into one nostril straight back (not upwards) and continue along the floor of the nasal passage until reaching the nasopharynx. Rotate it several times to obtain secretions. Remove the swab and insert the swab into the specimen collection tube. Break the swab shaft and leave the swab in the tube. Swab specimens to be tested can be stored for 1 day at room temperature, 4 days at 2-8°C, and long-term storage below −20°C.

- **Sputum**

  Collect sputum specimen by inducing a cough into a sterile container. Sputum specimens to be tested can be stored for 1 day at room temperature, 4 days at 2-8°C, and long-term storage below −20°C.

⚠ Only use preservative-free containers.

⚠The clinical samples can be stored up to a day at 2 – 8 °C. For longer period of storage, the clinical samples should be stored at −80 ~ −20 °C in aliquots.

7.5 Sample pretreatment

- **Swab**

1) Vortex vigorously that viruses on the swab are detached well into the solution (Transport buffer or 1x PBS).
2) Transfer 200 ㎕ of the sample to an 1.5 ㎖ microcentrifuge tube for ensuing nucleic acid extraction.

- Sputum

1) Add equal amount of E-z Solution to Sputum and vortex vigorously.
   ※ E-z Solution can be directly added to sputum collection tube.
2) Incubate at room temperature for 10 min.
   ※ Check the viscosity of sample. DO NOT proceed to nucleic acid extraction when the sample is still viscous. If the sample is still viscous, add more E-z Solution to the sample. Incubate the sample at room temperature until it is turned into a liquid form.

7.6 Before you proceed, check the followings.

✓ Completely dissolve Proteinase K in 1,250 µl of ER Buffer. Dissolved Proteinase K should be stored at 4℃ at least 1 week. For long term use, aliquot the dissolved Proteinase K and store at −20℃.

✓ Dissolve Poly (A) with 500 µl of ER Buffer and gently mix with vortex mixer. Mix dissolved Poly (A) solution into VB buffer and shake it thoroughly.

✓ Add adequate amount of absolute ethanol to VW1 Buffer. Please note the instruction on label of VW1 Buffer.

✓ Before starting extraction process, heat the ER Buffer at 56 – 60°C.

✓ To confirm whether the nucleic acid extraction process has been performed normally, the use of internal control RNA is recommended. Internal control RNA is not supplied with this product, and add the adequate amount of internal control RNA to the sample by referring to the instruction provided by the manufacturer of the downstream assay.

※ VB Buffer contains irritant chaotropic salt. Take appropriate laboratory safety precaution, and wear gloves when handling.

8. Warning and precaution

VB Buffer

Risk·Hazard Classification:

Acute toxicity (oral): Class 3

Specific target organ toxicity (single exposure) : Class 3 (Respiratory system irritation)

Skin corrosion/irritation: Class 2

Extreme eye damage/eye irritation : Class 1

Chronic aquatic hazard : Class 4

Risk·Hazard phrase: H302, H315, H319, H335, H413

Prevention: P261, P264, P270, P271, P273, P280

Response: P301+ P310, P302+ P352, P304+P340, P305+P51+P338, P310, P312, P330, P332+P313, P362+ P364

Storage: P403+P235, P405

Disposal: P501

VW1 Buffer

Risk·Hazard Classification:

Acute toxicity (oral): Class 4

Specific target organ toxicity (single exposure) : Class 3 (Respiratory system irritation)

Skin corrosion/irritation: Class 2

Extreme eye damage/eye irritation : Class 2

Chronic aquatic hazard : Class 4

Risk·Hazard phrase: H302, H315, H319, H335, H413

Prevention: P261, P264, P270, P271, P273, P280

Response: P301+ P312, P302+ P352, P304+P340, P305+P51+P338, P312, P330, P332+P313, P337+P313, P362

Storage: P403+P235, P405

Disposal: P501

RWA2 Buffer

	Risk·Hazard Classification : Flammable liquids : Catergory 2 Serious eye damage/eye irritant : Category 2 Cell mutagenicity : Category 1B Carcinogenicity : Category 1A Risk·Hazard phrase : H225, H319, H340, H350 Prevention : P201,P202, P210, P233, P240, P241, P242, P243, P264, P280 Response : P303+P361, P305+P351+P338, P308+P313, P337+P313, P370+P378 Storage : P403+P235, P405 Disposal : P501

ER Buffer

	No hazardous ingredient

Proteinase K

	Risk·Hazard Classification : Specific target organ toxicity (single exposure) : Class 3 (Respiratory system irritation) Skin corrosion/irritation: Class 2 Extreme eye damage/eye irritation : Class 2 Risk·Hazard phrase : H315, H319, H335 Prevention : P261, P264, P271, P280 Response : P302+P352, P304+P340, P305+P351+P338, P312, P332+313, P337+P313, P362+P364 Disposal : P501

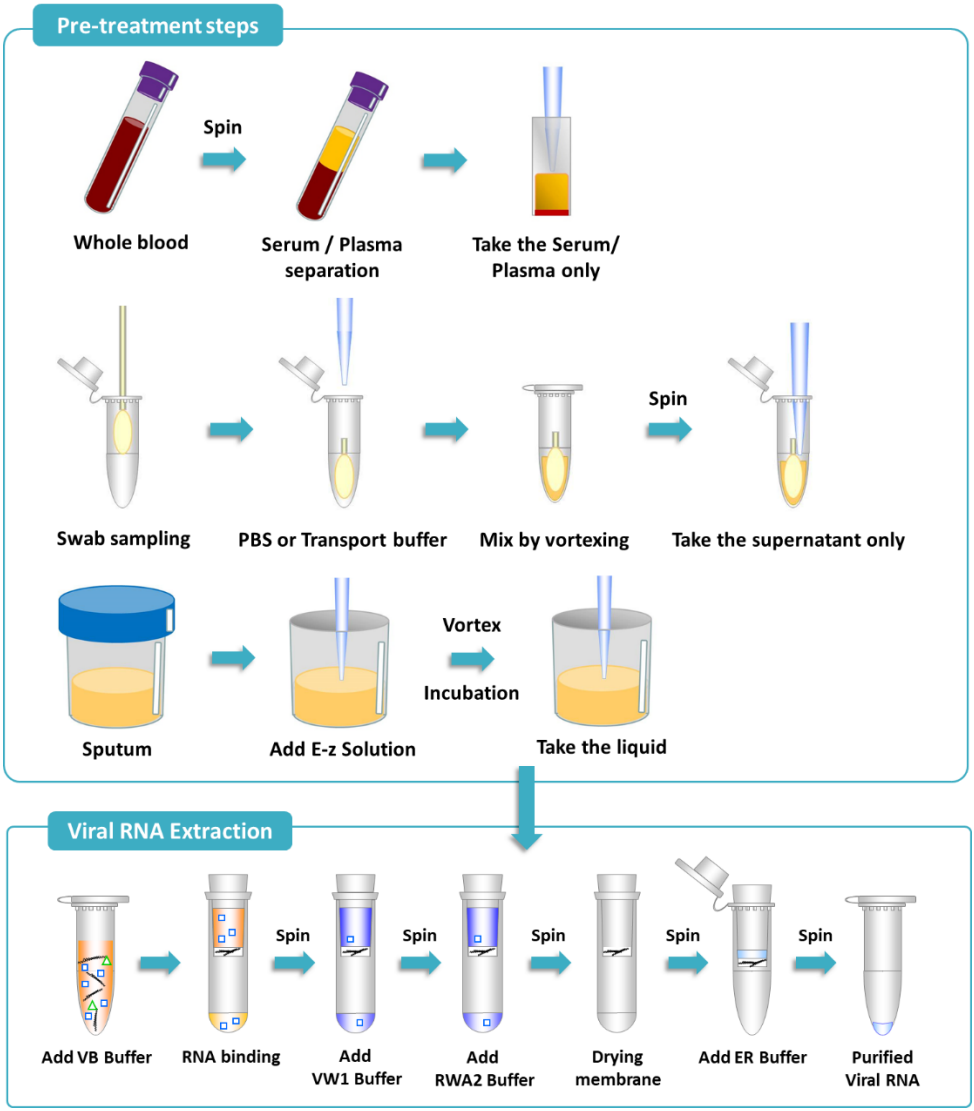
Poly A

	No hazardous ingredient

BST Solution

	Risk·Hazard Classification : Flammable liquids: Category 2 Metallic corrosive substances : Category 1 Acute toxicity (oral): Category 4 Acute toxicity (skin): Category 4 Eye irritation: Category 2A Risk·Hazard phrase : H225, H290, H302, H315, H319, H312, H350 Prevention : P201,P202, P210, P233, P234, P240, P241, P242, P243, P264, P270, P280 Response : P310, P321, P301+P312, P303+P361, P302+P352, P305+P351+P338, P308+P313, P312, P330, P332+P313, P362+P364, P370+P378, P390 Storage : P402+P404, P405, P406 Disposal : P501

9. Experimental protocol



Viral RNA Extraction

1) Add 10 µl of Proteinase K to a 1.5 ml or 2 ml tube.

2) Add 200 µl of sample (serum, plasma, swab or sputum) to the tube.
See **7.4 Sample collection and storage** on page 5 and **7.5 Sample pretreatment** on page 6.

3) Add 300 µl of VB buffer in the tube and mix by vortexing for 10 sec.
To ensure efficient lysis, the sample should be mixed thoroughly with VB Buffer.

4) Incubate at 56 – 60℃ for 10 min.

5) Add 300 µl of isopropanol, lightly vortex for about 10 sec. Spin down for 5 sec to down the lysate clinging to the walls and lid of the tube.

6) Add 100 µl of BST Solution to the binding column tube (fit in a collection tube) and centrifuge for 30 sec at 13,000 rpm.

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

8) Transfer the lysate into the binding column tube not getting the lid wet.

9) Close the tube and centrifuge at 13,000 rpm for 1 min.
If the lysate has not completely passed the column following centrifugation, then centrifuge again until the liquid completely passes through.

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

11) Add 500 µl of VW1 Buffer to the column, close the lid, and centrifuge for 1 min at 13,000 rpm.

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

13) Add 600 µl of RWA2 Buffer, close the lid, and centrifuge for 1 min at 13,000 rpm.

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

15) Centrifuge once more at 13,000 rpm for 1 min to remove ethanol completely.

Make sure that there is no droplet hanging from the bottom of the binding column. Residual RWA2 Buffer left in the binding column may cause problems in later applications.

16) Transfer the Binding column to a 1.5 ml tube for elution, add 50 µl of ER Buffer, and wait for 1 minute to allow the buffer to permeate the column.

We recommend letting stand for about 5 min to increase RNA yield. You can also increase yield by heating the ER Buffer at about 60℃ before adding to the column.

17) Elute by centrifuge at 13,000 rpm for 1 min. The eluted RNA solution can directly be used, or stored at −70℃ for longer storage.

10. Troubleshooting

1) Yield or purity of RNA is low

- ✓ The kit may have been stored under non-optimal conditions.
  Store kit at 15 – 35℃ at all times upon arrival.

- ✓ Mix VB Buffer completely after adding Poly (A).

- ✓ Ethanol may not have been added to VW1 Buffer.
  Add absolute ethanol to VW1 Buffer before using. After adding ethanol, mix the VW1 Buffer well and store at 15 – 35℃. Always mark the VW1 Buffer vial to indicate whether ethanol has been added or not.

- ✓ Reagents and samples may not have been completely mixed.
  Always mix the sample tube thoroughly after adding each reagent.

2) Absorbance at 260 nm is very high

- ✓ VB Buffer of 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 product can show high absorbance at 260 nm UV. For quantification of extracted viral RNA, amplification is recommended.

11. References

N. J. Coombs. *et al.* (1999) *Nucleic Acids Res.,* Vol **27**, No.16

C. Reno. *et al.* (1997) *Biotechniques* , Vol **22**, No. 6

Michael J.Bonham. *et al.* (1996) *Biotechniques,* Vol **20**, No. 5

12 Explanation of symbol

	Catalog Number		Contains sufficient for (n) tests
	USE BY		Temperature Limitation
	Batch code		Caution
	Manufacturer		Biological risks
	DO NOT REUSE		Consult Instructions For Use
	In Vitro Diagnostics Medical Device		Conformite Europeenne Mark
	Authorized representative in the European Community		

Bioneer Worldwide

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