Cat. # 9766

For Research Use

TaKaRa

TaKaRa MiniBEST Viral RNA/DNA Extraction Kit Ver.5.0

Product Manual

v201408Da

Table of Contents

I. Description

TaKaRa MiniBEST Viral RNA/DNA Extraction Kit Ver.5.0 is designed to purify virus RNA/DNA from a variety of sample sources including plasma, whole blood, cell-free body fluids, virus stock solution, and infected tissue. This system employs a special lysis buffer in combination with nucleic acid preparation membrane to efficiently purify viral RNA/DNA from the biological sample. The protocol provides a simple method to achieve the rapid isolation of highly purified RNA/DNA and to be accomplished within 20 minutes after virus lysis. RNA can be extracted from 1.0 x 10^4 copies of Hepatitis A virus and 1.6 x 10^5 copies of the Flury strains of rabies virus etc. RNA prepared is suitable for a variety of applications, such as RT-PCR, Northern blotting and other molecular biology experiments.

II. Components (50 reactions)

The kit contains part I and part II.

Part I (stored at −20℃)

Proteinase K (20 mg/ml)	1 ml
Carrier RNA	50 μl

Part II (stored at room Temperature (15 - 25℃))

Buffer VGB＊1	12 ml
Buffer RWA＊1	28 ml
Buffer RWB＊2	24 ml
RNase free dH_2O	2 ml x 2
Spin Columns	50
Collection tubes	50
RNase free collection tube (1.5 ml)	50

＊1: Contains Strong denaturant. Be careful to avoid contacting with skin and eyes. In the case of such contact, wash immediately with plenty water and seek medical advice.

＊2: Before using the kit, add 56 ml of 100% ethanol. Mix well.

Reagents not supplied in this kit

1. 100% ethanol
2. PBS solution

III. Storage and shipping

1. Part I can be stored at −20℃ , and part II of the kit can be stored at room temperature (15 - 25℃).
2. Part I can be shipped at −20℃ , and part II of the kit can be shipped at room temperature (15 - 25℃).

IV. Preparation before experiment

1. Adjust a water bath to 56℃ .
2. Add 56 ml of 100% ethanol to Buffer RWB and mix well.
3. When eluting RNA, please use RNase free dH_2O.

V. Protocol

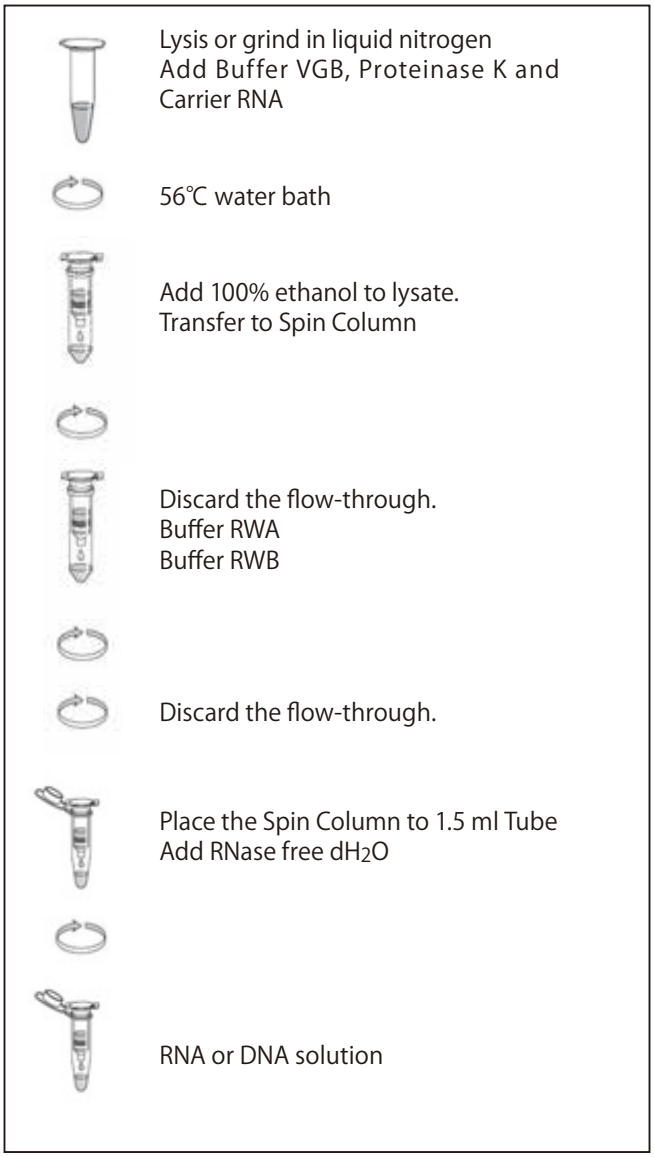


Figure 1. Flow chart

Protocol overview is in Figure 1. The procedure after virus lysis can be accomplished in 20 minutes. (If the starting material is the virus infected tissue, need to grind the tissue in liquid nitrogen.) The whole procedure includes lysis of virus, RNA/DNA binding with column and RNA/DNA purification. Protocol in detail is as below.

1. Lysis of virus
   Protocols are provided for efficient lysis, and homogenization of each sample type.

   ● Lysis of virus in plasma, serum, cell-free body fluids and the virus stock solution
   (1) Collect 10 - 200 μl of plasma, serum,cell-free body fluids and the virus stock solution. If the starting sample is less than 200 μl, add PBS solution or RNase free dH_2O to make up to 200 μl.
   (2) Add 200 μl of Buffer VGB, 20 μl of Proteinase K and 1.0 μl of Carrier RNA.Mix well and incubate it into 56℃ water bath for 10 minutes.
   (3) Add 200 μl of 100% ethanol to the lysate and mix well.

   ● Lysis of the virus infected tissue
   (1) Collect 10 mg virus infected tissue to grind in liquid nitrogen. Add 200 μl PBS solution or RNase free dH_2O.
   (2) Add 200 μl of Buffer VGB, 20 μl of Proteinase K and 1.0 μl of Carrier RNA. Mix well and incubate it into 56℃ water bath for 10 minutes.
   (3) Add 200 μl of 100% ethanol to the lysate and mix well.

2. Transfer the solution to Spin Column with Collection tube. Centrifuge at 12,000 rpm for 2 minutes. Discard the flow-through.

3. Add 500 μl of Buffer RWA into Spin Column. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.

4. Add 700 μl of Buffer RWB into Spin Column. Take care to add Buffer RWB along the tube wall of Spin Column to wash off any residual salt. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.

   Note : Make sure the amount of 100% ethanol specified on the bottle label has been added to the Buffer RWB.

5. Repeat Step 4.

6. Place Spin Column into Collection Tube. Centrifuge at 12,000 rpm for 2 minutes.

7. Place Spin Column into a new 1.5 ml RNase free collection tube. Add 30 - 50 μl of RNase free dH_2O to the center of the membrane. Let it stand for 5 minutes at room temperature.

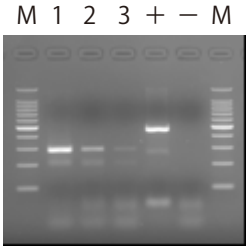
   Note : When eluting RNA, please use RNase free dH_2O.

8. Centrifuge at 12,000 rpm for 2 minutes to elute RNA/DNA. If more yield is needed, the flow-through can be re-added into the center of the membrane or add 30 - 50 μl of RNase free dH_2O and let it stand for 5 minutes at room temperature and centrifuge at 12,000 rpm for 2 minutes to elute RNA/DNA.

9. Quantification of the RNA/DNA. Since Carrier RNA is added, RNA/DNA extracted cannot be quantitatively determined by electrophoresis or absorbance measuring. RNA/DNA can be determined by PCR, RT-PCR, qPCR.

VI. Experimental examples

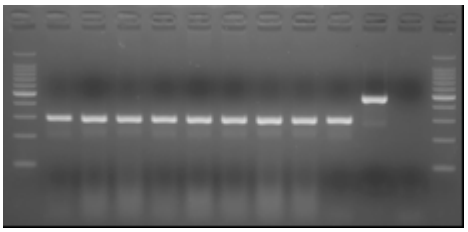
1. Purification of HAV RNA from hepatitis A vaccine stock solution and detected by RT-PCR
   RNA was extracted from 1 μl, 0.1 μl, 0.01 μl of hepatitis A vaccine stock solution (equivalent to 6.0 x 10^6, 6.0 x 10^5, 6.0 x 10^4 copies of HAV) and detected by RT-PCR with TaKaRa One Step RNA PCR Kit (AMV) (Cat. #RR024A) to detect 289 bp DNA fragment. The electrophoresis is in Figure 2.



1% Agarose gel electrophoresis
M : 100 bp DNA Ladder (Dye Plus)
1 : 6.0 x 10^6 HAV
2 : 6.0 x 10^5 HAV
3 : 6.0 x 10^4 HAV
＋/－: positive/negative control (Cat. #RR024A)

Figure 2. Purification of HAV RNA from hepatitis A vaccine stock solution and detection by RT-PCR

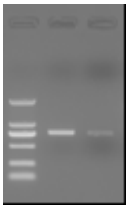
2. Purification of HAV RNA from HL60 cell culture medium, whole blood, saliva, urine, mouse liver
   HAV RNA was extracted from 200 μl of cultured cell (HL60), 10 μl of whole blood, 200 μl of saliva, 200 μl of urine, 10 mg of mouse liver and detected by RT-PCR with TaKaRa One Step RNA PCR Kit (AMV) (Cat. #RR024A) to detect 289 bp DNA fragment. The electrophoresis is in Figure 3.



1% Agarose gel electrophoresis
M : 100 bp DNA Ladder (Dye Plus)
1.2 : HL60 cell culture medium
3.4 : whole blood
5.6 : saliva
7.8 : urine
9 : mouse liver
＋: Positive control (Cat. #RR024A)
－: Negative control (Cat. #RR024A)

Figure 3. Purification of HAV RNA from HL60 cell culture medium, whole blood, saliva, urine, mouse liver

3. Purification of RNA from Flury strain of rabies vaccine and detection by RT-PCR.
   RNA has been extracted from 10 μl and 0.1 μl of Flury strain of rabies vaccine and detected by RT-PCR with TaKaRa One Step RNA PCR Kit(AMV) (Cat. #RR024A) to detect 500 bp DNA fragment. The detection sensitivity is 1.6 x 10^7 and 1.6 x 10^5. The electrophoresis is in Figure 4.



1% Agarose gel electrophoresis
M : DL2,000 DNA Marker
1 : 1.6 x 10^7 Flury strain of rabies vaccine
2 : 1.6 x 10^5 Flury strain of rabies vaccine

Figure 4. Purification of RNA from Flury strain of rabies vaccine and detection by RT-PCR

VII. Cautions

1. Try to use fresh experimental material to ensure the extracted genomic DNA or RNA can't be degraded.
2. When using liquid nitrogen grinding tissue material, liquid nitrogen should be added at any time to ensure that the extracted RNA is not degraded.
3. Some reagents contain chemical irritants. When working with these reagents, always wear suitable protection such as safety glasses, laboratory coat and gloves and try to operate at fume hood. Be careful to avoid contacting with eyes and skin. In the case of such contact, wash immediately with plenty water and seek medical advice.
4. RNase free dH_2O should be used for elution of RNA.
5. The Carrier RNA in the kit is RNA obtained from *Escherichia coli*. It is used to enhance binding of viral nucleic acids to the membrane and reduce the risk of viral RNA degradation. When the purified DNA/RNA is used as PCR template, we recommend that the sequences of PCR primers are compared with *E. coli* genome sequences. Because PCR become false positive when the primer sequence has high homology with the Carrier RNA, the primers should be re-designed. To test whether the designed PCR primers can cross-react with Carrier RNA, you can examine by RT-PCR using the primers and Carrier RNA. If any PCR product appear by RT-PCR, the primer sequence is highly homologous with the Carrier RNA. If not use Carrier RNA, the yield of DNA/RNA will be reduced.

VIII. Maximum amount of starting materials

Material	Maximum amount of starting
Cell culture medium	200 μl
Nucleated whole blood	10 μl
Nonnucleated whole blood	200 μl
Plasma	200 μl
Cell-free body fluids	200 μl
Virus stock	200 μl
Virus infected tissue	10 mg

IX. Troubleshooting

Q1. How yield of virus RNA/DNA is extracted?

A1. This kit can extract RNA/DNA from 6.0 x 10^4 of the HAV and 1.0 x 10^5 of Flury strains of rabies virus.

Q2. What range of applications of this kit ?

A2. This kit can be used to purify viral RNA/DNA from a wide range of sample sources, such as plasma, whole blood, no cell body fluids, virus stock and virus infected tissue.

Q3. If the extracted nucleic acid have low biological activity.

A3. (1) The salt concentration in extracted nucleic acid is too high. In washing step of the membrane with Buffer RWA and Buffer RWB, add the buffer along the tube wall of Spin Column for improving the washing effect.
(2) There is residual ethanol in DNA or RNA Solution. Let the column stand for 2 minutes at room temperature before adding Elution Buffer to the column and it will improve the effect of elution.
(3) The RNase free dH_2O for elution must be added in the center of membrane and not residue on the tube wall of Spin Column.