Cat. # 9766

For Research Use

TaKaRa

TaKaRa MiniBEST Viral RNA/DNA Extraction Kit Ver.5.0

Product Manual

v202005Da

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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 solution, and infected tissue. This system employs a special lysis buffer in combination with nucleic acid binding membrane to efficiently purify viral DNA or RNA from DNA or RNA virus in 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. Viral RNA purified 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 Package I and II.

Package I

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

Package II

Buffer VGB＊1		12 ml
Buffer RWA＊1		28 ml
Buffer RWB＊2		24 ml
RNase free dH_2O		2 ml x 2
Spin Column		50
Collection tube		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 96 - 100% ethanol. Mix well.

Materials Required but not Provided

1. 96 - 100% ethanol
2. PBS solution

III. Storage

Package I : -20℃

Package II : Room temperature (15 - 25℃)

IV. Preparation before experiment

1. Add 56 ml of 96 - 100% ethanol to Buffer RWB and mix well.
2. Adjust a water bath to 56℃.

V. Precautions

1. Use fresh experimental material to ensure the extracted viral DNA or RNA can't be degraded.
2. When using liquid nitrogen for grinding tissue material, liquid nitrogen should be added at any time to ensure that 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 recovery of viral nucleic acids with Spin Column 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.

VI. Protocol

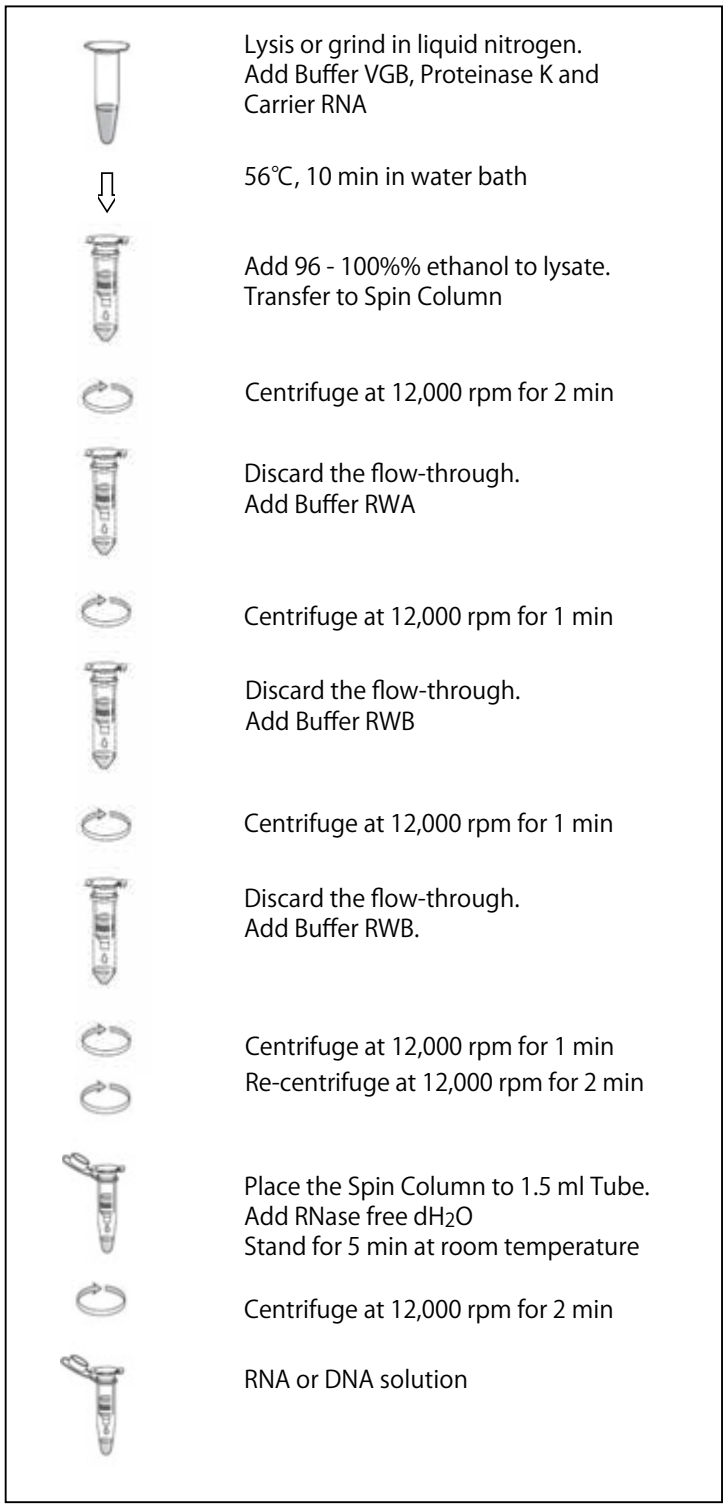


Figure 1. Protocol overview

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.

1. Sample preparation
   Protocols are provided for efficient lysis and homogenization of each sample.

   ● Preparation of plasma, serum, cell-free body fluids, and virus stock solution
   (1) Collect 10 - 200 μl of plasma, serum, cell-free body fluids, or 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 at 56℃ water bath for 10 minutes.
   (3) Add 200 μl of 96 - 100% ethanol and mix well to prepare the lysate.

   ● Preparation of the virus infected tissue
   (1) Collect 10 mg virus infected tissue grinded 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 at 56℃ water bath for 10 minutes.
   (3) Add 200 μl of 96 - 100% ethanol and mix well to prepare the lysate.

2. Transfer the lysate to Spin Column with Collection tube. Centrifuge at 12,000 rpm for 2 minutes. Discard the flow-through. And re-set Spin Column into the Collection tube.

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

4. Add 700 μl of Buffer RWB (with 96 - 100% ethanol) into Spin Column. Take care to add Buffer RWB along the wall of Spin Column to wash off any residual salt. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through. And re-set Spin Column into the Collection tube.

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

5. Repeat Step 4.

6. Place Spin Column into the Collection Tube. Re-centrifuge at 12,000 rpm for 2 minutes to dry the column membrane.

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 in the Spin Column. Let it stand for 5 minutes at room temperature.

   Note : When eluting RNA, use RNase free dH_2O.

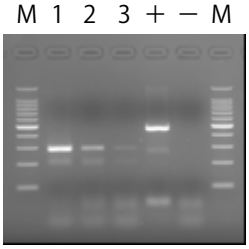
8. Centrifuge at 12,000 rpm for 2 minutes to elute RNA or DNA. If more yield is needed, add 30 - 50 μl of RNase free dH_2O and let it stand for 5 minutes at room temperature and centrifuge at 12,000 rpm for 2 minutes to elute RNA or DNA. And combine two eluted solution.

Note: Since Carrier RNA is added, RNA or DNA extracted cannot be quantitatively determined by absorbance.

VII. Experimental examples

1. Purification of Hepatitis A virus RNA

HAV RNA was extracted from 1 μl, 0.1 μl, 0.01 μl of hepatitis A vaccine 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). The 289 bp DNA fragment was detected.

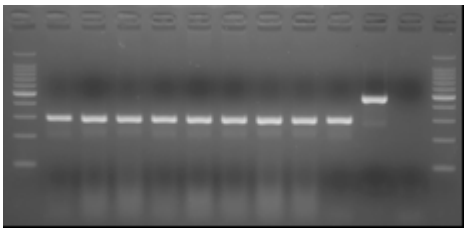


1% Agarose gel electrophoresis
M : 100 bp DNA Ladder (Dye Plus)
1 : 6.0 x 10^6 HAV
2 : 6.0 x 10^5 HAV
3 : 6.0 x 10^4 HAV
＋ : Positive Control (Cat. #RR024A)
－ : H_2O

Figure 2.

2. Purification of viral RNA from HAV in cell culture medium, whole blood, saliva, urine, or mouse liver

HAV was added in 200 μl of HL60 culture medium, 10 μl of whole blood, 200 μl of saliva, 200 μl of urine, or 10 mg of mouse liver. HAV RNA were purified from these samples and detected by RT-PCR with TaKaRa One Step RNA PCR Kit (AMV) to detect 289 bp DNA fragment.

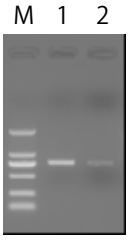


1% Agarose gel electrophoresis
M : 100 bp DNA Ladder (Dye Plus)
1.2 : HL60 culture medium
3.4 : whole blood
5.6 : saliva
7.8 : urine
9 : mouse liver
＋ : Positive Control (Cat. #RR024A)
－ : H_2O

Figure 3. Purification of HAV RNA from various samples

3. Purification of rabies virus RNA

Virus RNA has been extracted from 10 μl and 0.1 μl of Flury strain of rabies vaccine (equivalent to 1.6 x 10^7 and 1.6 x 10^5 copies of rabies virus) and detected by RT-PCR with TaKaRa One Step RNA PCR Kit(AMV) to detect 500 bp DNA fragment. .



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

VIII. Maximum amount of starting materials

Material	Maximum amount of starting
Cell culture medium	200 μl
Whole blood	100 μl
Serum	200 μl
Plasma	200 μl
Cell-free body fluid	200 μl
Virus solution	200 μl
Virus infected tissue	10 mg

IX. Troubleshooting

<If the extracted nucleic acid have low quality.>

(1) The salt concentration in extracted nucleic acid is too high. In washing step of the membrane with Buffer RWA and 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 RNase free dH_2O to the column.
(3) No elution from membrane. RNase free dH_2O must be added in the center of membrane.