AllPure Viral DNA/RNA Extraction Kit

Cat. No. ABTGNA016-50

Storage: Carrier RNA and Proteinase K solution at -20°C for one year; others at room temperature for one year

Description

AllPure Viral DNA/RNA Extraction Kit provides a simple and fast column based method to isolate viral RNA/DNA from up to 200 µl of plasma, serum, body fluid and mammalian cell supernatant. Samples are lysed with unique lysis buffer and RNA is enriched by carrier RNA. DNA/RNA is bound to silica-membrane. After washing, high quality DNA/RNA is eluted from the column. RNA/DNA is free of protein contamination, and is suitable for PCR, RT-PCR, qRT-PCR and qPCR.

Kit Contents

Component	50 rxns
Binding Buffer 5 (BB5)	15 ml
Wash Buffer 5 (WB5)	12 ml
Proteinase K (20 mg/ml) -20°C	1 ml
RNase-free Water	10 ml
RNase-free Tube (1.5 ml)	50 each
RNA Spin Columns with Collection Tubes	50 each

Sample requirement

- Storage: at 2-8°C for 4 hours; at -20°C or -80°C for long term storage.
- Avoid repeated freezing and thawing for plasm and serum (no more than once)

Procedure

Before starting, add 48 ml of 96%-100% ethanol to the 12 ml of WB5, mix thoroughly.

Prepare BB5 with carrier RNA according to the table given in next page.

All centrifugation steps were carried out at room temperature.

1. Add 20 µl of Proteinase K to a sterile 1.5 ml microcentrifuge tube.
2. Add 200 µl of sample to tube. (Note: If the sample volume is less than 200 µl , please add PBS or 0.9% NaCl to 200 µl).
3. Add 200 µl of BB5 with carrier RNA, mix by vortexing for 15 seconds.
4. Incubate at 56°C for 15 minutes.
5. Add 250 µl of 96%-100% ethanol to the sample (the mixture may appear floculation at this stage). Mix by vortexing for 15 seconds and incubate at room temperature (15-25°C) for 15 minutes.
6. Transfer the entire contents to a spin column and centrifuge at 12,000×g for 1 minute, discard flow through.
7. Add 500 µl of WB5 (check to make sure that ethanol has been added). Centrifuge at 12,000×g for 1 minute, discard the flow through.
8. Repeat step 7 once.
9. Centrifuge at 12000×g for 1 minute to remove residual ethanol and air-dry the membrane completely.
10. Place the spin column into a clean RNase-free 1.5 ml microcentrifuge tube. Add 20-50 µl of RNase-free Water to the center of the column, and incubate at room temperature for 1 minute.
11. Centrifuge at 12000×g for 1 minute.
12. Store the eluted DNA (at -20°C) or RNA (at -80°C).

AllBio Science, Inc www.allbioscience.com Tel：+886 988-628969 E-maill：service@allbioscience.com

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Addition of carrier RNA to BB5

Calculate the volume of BB5 with carrier RNA needed by selecting the number of samples to be processed from Table 1.

Gently mix by inverting the tube 10 times. To avoid foaming, do not vortex.

For larger numbers of samples, volumes can be calculated using the following sample calculation:

N × 0.21 ml = A ml

A ml × 28 µl/ml = B µl

where: N = number of samples to be processed simultaneously

A = volume of BB5 needed

B = volume of carrier RNA to be added to BB5 needed

Table 1. Volumes of BB5 and carrier RNA required for different number of samples.

N	A	B	N	A	B
1	0.21 ml	5.9 µl	6	1.26 ml	35.4 µl
2	0.42 ml	11.8 µl	7	1.47 ml	41.3 µl
3	0.63 ml	17.7 µl	8	1.68 ml	47.2 µl
4	0.84 ml	23.6 µl	9	1.89 ml	53.1 µl
5	1.05 ml	29.5 µl	10	2.1 ml	59 µl

Notes

- All the centrifugation steps are carried out at room temperature.
- Please check to make sure that 96%-100% ethanol has been added into WB5 before use.
- Aliquot the Carrier RNA into RNase-free microcentrifuge tubes and store at -20°C. Avoid repeated freezing and thawing (not more than three times) for Carrier RNA.