EasyPrep Virus DNA/RNA Kit

For simultaneous purification of viral RNA and DNA from plasma, serum, and cell-free body fluids

Contents

Introduction

EasyPrep Virus DNA/RNA Kit provides a fast, simple, and cost-effective viral DNA/RNA miniprep method, and it is suitable for viral RNA and DNA from plasma, serum, and cell-free body fluids. This product uses silica membrane technology and thus avoids the cumbersome steps associated with loose resins or slurries. Viral DNA/RNA purified with the EasyPrep Virus DNA/RNA Kit is immediately ready for use. Phenol extraction and ethanol precipitation are not required. The purified DNA/RNA is ready for use in downstream applications such as enzymatic reactions, RT–PCR, and Southern blotting.

Important Note:

1. All protocol steps should be carried out at room temperature (15–25 °C).
2. Equilibrate the samples to room temperature.
3. RNase-free microcentrifuge tubes (1.5 mL) are used in step 13. Others are not supplied.

Preparation of Carrier RNA solutions

1. Buffer GBD is supplied as a concentrate. Before using for the first time, add 17 mL ethanol (96%–100%), to obtain a working solution.
2. Add 310 µL of RNase-free ddH_2O to the tube containing 310 µg of lyophilized carrier RNA to obtain a solution of 1 µg/µL. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at −20 °C. Do not freeze–thaw the aliquots of carrier RNA more than three times.
3. Carrier RNA cannot be dissolved in Buffer GBB directly. It must first be dissolved in RNase-free ddH_2O and then added to Buffer GBB.
4. Carrier RNA working solution: Calculate the volume of Buffer GBB/Carrier RNA mix required per batch of samples by selecting the number of samples to be simultaneously processed from Table 1.

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

n × 0.22 mL = y mL

y mL × 28 µL/mL = z µL

n = number of samples to be processed simultaneously

y = calculated volume of Buffer GBB

z = volume of carrier RNA/RNase-free ddH_2O to add to Buffer GBB

Table 1 Volumes of Buffer GBB and Carrier RNA/RNase-free ddH_2O Mix required for the Procedure

No. Sample	Vol. Buffer GBB (mL)	Vol. Carrier RNA/ RNase-free ddH_2O (µL)
1	0.22	6.2
2	0.44	12.3
3	0.66	18.5
4	0.88	24.6
5	1.10	30.8
6	1.32	37.0
7	1.54	43.1
8	1.76	49.3
9	1.98	55.4
10	2.20	61.6
11	2.42	67.8
12	2.64	73.9
13	2.86	80.1
14	3.08	86.3
15	3.30	92.4
16	3.52	98.6
17	3.74	104.7
18	3.96	110.9
19	4.18	117.0
20	4.40	123.2
21	4.62	129.4
22	4.84	135.5
23	5.06	141.7
24	5.28	147.8

Note: Mix Buffer GBB with carrier RNA solution upside down. Do not vortex to avoid bubbling.

Kit Contents

Contents	DPT-BC15 (50 preps)
Buffer GBB	15 mL
Buffer GBD	13 mL
Buffer RBW	15 mL
RNase-free ddH2O (Bottled)	15 mL
Proteinase K	1 mL
Carrier RNA	310 μg
RNase-free ddH2O (Tubular)	1 mL
RNase-free Spin Columns CR2	50
RNase-free Microcentrifuge Tubes (1.5ml)	50

Storage

1. All buffers should be stored at room temperature (15–25 °C).
2. Lyophilized carrier RNA can be stored at room temperature (15–25 °C) until the expiration date shown on the kit box. Carrier RNA should be dissolved in RNase-free ddH_2O and stored at −20 °C. Dissolved carrier RNA should be added to Buffer GBB as described. This solution should be prepared fresh and is stable at 2–8 °C for up to 48 h.

Protocol

1. Pipette 20 μL proteinase K into a clean 1.5-mL microcentrifuge tube (not provided).
2. Add 200 μL of plasma or serum to the microcentrifuge tube (equilibrate the samples to room temperature.).

Note: If the sample volume is <200 μL, add the appropriate volume of 0.9% sodium chloride solution to bring the volume of protease and sample to 220 μL.

3. Add 200 μL of Buffer GBB (containing 28 μg/mL Carrier RNA).

Close the cap and mix through pulse-vortexing for 15 sec.

Note: To ensure efficient lysis, it is essential that the sample and Buffer GBB are mixed thoroughly to yield a homogeneous solution..

4. Incubate at 56 °C for 15 min in a heating block. Briefly centrifuge the 1.5-mL tube to remove drops from the inside of the lid.
5. Add 250 µL of ethanol (96%–100%) to the sample (precipitates may be visible after adding ethanol), close the cap, and mix thoroughly through pulse-vortexing for 15 sec. Incubate the lysate with ethanol for 5 min at room temperature (15–25 °C).

Note: Cool ethanol (96%–100%) on ice before use if the room temperature is >25 °C.

6. Briefly centrifuge the 1.5-mL microcentrifuge tube to remove drops from the inside of the lid.
7. Carefully transfer the lysate, including any precipitate that may have formed, onto the RNase-free Spin Columns CR2 in a 2-mL RNase-free Collection Tube without wetting the rim. Close the cap and centrifuge at 8,000 rpm (~6,000 ×g) for 1 min. Discard the filtrate; place the spin column in the same Collection Tube.

Note: If the lysate has not completely passed through RNase-free Spin Column CR2 after centrifugation, centrifuge again at higher speed until the spin column is empty.

8. Carefully open RNase-free Spin Column CR2, and add 500 µL of Buffer GBD (Ensure that ethanol (96%−100%) has been added into Buffer GBD before use) without wetting the rim. Close the cap and centrifuge at 8,000 rpm (~6,000 ×g) for 1 min. Discard the filtrate and place the spin column in the same Collection Tube.
9. Carefully open the RNase-free Spin Columns CR2, and add 600 µL of Buffer RBW (Ensure that ethanol (96%−100%) has been added into Buffer RBW before use) without wetting the rim. Close the cap, let it stand still for 2 min, and centrifuge at 8,000 rpm (~6,000 ×g) for 1 min. Discard the filtrate, and place the spin column in the same Collection Tube.
10. Repeat step 9.
11. Carefully open the RNase-free Spin Column CR2 and add 500 µL of ethanol (96%−100%) without wetting the rim. Close the cap and centrifuge at 8,000 rpm (~6,000 ×g) for 1 min. Discard the filtrate.

Note: Ethanol carries over into the elute may cause problems in downstream applications.

12. Place RNase-free Spin Column CR2 in the same Collection Tube. Centrifuge at full speed 12,000 rpm (~13,400 ×g) for 3 min to dry the membrane completely.
13. Place RNase-free Spin Columns CR2 in a clean 1.5-mL RNase-free microcentrifuge tube, and discard the Collection Tube with the filtrate. Carefully open the lid of the spin column, and apply 20–150 µL of RNase-free ddH_2O to the center of the membrane. Close the lid, and incubate at room temperature (15–25 °C) for 5 min. Centrifuge at full speed (12,000 rpm; ~13,400 ×g) for 1 min.

Note: Ensure that the elution buffer is equilibrated to room temperature (15–25 °C). If elution is performed in small volumes (<50 µL), the elution buffer must be dispensed onto the center of the membrane for complete elution of bound RNA and DNA.