EasyPrep Virus RNA Kit

For purification of viral RNA from plasma, serum, cell-free body fluids, or cell-culture supernatants

Contents

Introduction

The EasyPrep Virus RNA Kit provides the fastest and easiest method of purifying viral RNA for reliable use in amplification technologies. It is suitable for purification of viral RNA from plasma, serum, and cell-free body fluids, and the procedure is optimized for use with samples of 140–560 µL. Addition of carrier RNA enables purification of viral RNA from extremely small samples.

Silica membrane technology eliminates the cumbersome steps associated with loose resins or slurries. Viral RNA purified with the EasyPrep Virus RNA Kit is immediately ready for use in downstream applications such as enzymatic reaction, PCR, and southern blot.

Important Notes

1. Perform all protocol steps at room temperature (15–25 °C).
2. Equilibrate the samples to room temperature.
3. Prepare carrier RNA solutions as follows:

Add 310 µL of RNase-free ddH_2O to the tube containing 310 µg of lyophilized carrier RNA to obtain a solution concentration of 1 µg/µL. Dissolve the carrier RNA thoroughly, divide into conveniently sized aliquots, and store at –20 °C. Do not freeze and thaw the aliquots of carrier RNA more than three times. Do not dissolve lyophilized carrier RNA in Buffer LB directly. First dissolve carrier RNA in RNase-free ddH_2O, and then add it to the Buffer LB.

To prepare the carrier RNA working solution, calculate the volume of Buffer LB/carrier RNA mix required per batch of samples by selecting the number of samples to be simultaneously processed from Table 1. For large sample batches, volumes can be calculated using the following:

n × 0.56 mL = y mL

y mL × 10 µL/mL = z µL

n = number of samples to be processed simultaneously

y = volume of Buffer LB

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

4. Add ethanol (96%–100%) to the Buffer GBR and Buffer WB before use (volume is shown on the bottle packaged with the kit).

Kit Contents

Contents	DPT-BC15R (50 rxn)
Buffer LB	30 mL
Buffer GBR	13 mL
Buffer WB	12 mL
RNase-free ddH_2O (bottled)	15 mL
Carrier RNA	310 µg
RNase-free ddH_2O (tubular)	1 mL
RNase-free Spin Columns CR2 in 2-mL collection tubes	50
RNase-free microcentrifuge tubes (1.5 mL)	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 on the box. Carrier RNA should be dissolved in RNase-free ddH_2O and stored at −20 °C. Dissolved carrier RNA should be added to Buffer LB (Table1). This solution should be prepared fresh and is expected to remain stable at 2–8 °C for up to 48 hours.

Table 1 Volumes of Buffer LB and carrier RNA/RNase-free ddH_2O mix required for carrier RNA working solution

Samples	Buffer LB (mL)	Carrier RNA/RNase-free ddH_2O (µL)
1	0.56	5.6
2	1.12	11.2
3	1.68	16.8
4	2.24	22.4
5	2.80	28.0
6	3.36	33.6
7	3.92	39.2
8	4.48	44.8
9	5.04	50.4
10	5.60	56.0
11	6.16	61.6
12	6.72	67.2
13	7.28	72.8
14	7.84	78.4
15	8.40	84.0
16	8.96	89.6
17	9.52	95.2
18	10.08	100.8
19	10.64	106.4
20	11.2	112
21	11.76	117.6
22	12.32	123.2
23	12.88	128.8
24	13.44	134.4

Note: Mix Buffer LB with carrier RNA solution upside down. To avoid bubbling, do not form a vortex.

Protocol

1. Pipette 560 µL of Buffer LB containing carrier RNA (prepared according to Table 1 or the formula provided) into a clean 1.5-mL microcentrifuge tube.

Note: If the sample volume is larger than 140 µL, increase the volume of Buffer LB/carrier RNA proportionally (e.g., a 280-µL sample requires 1120 µL of Buffer LB/carrier RNA), and use a larger tube..

2. Add 140 µL of plasma, serum, or cell-free body fluid (equilibrated room temperature [15–25 °C]) to the Buffer LB/carrier RNA mixture in the microcentrifuge tube. Mix by pulse-vortexing for 15 s.

Note: To ensure efficient lysis, mixed the sample thoroughly with Buffer LB to yield a homogeneous solution.

3. Incubate at room temperature (15–25 °C) for 10 min.
4. Briefly centrifuge the tube to remove droplets formed inside the lid.
5. Add 560 µL of ethanol (96%–100%) to the sample, and mix by pulse-vortexing for 15 s.

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

6. Briefly centrifuge the tube to remove droplets formed inside the lid.
7. Carefully transfer 630 µL of lysate into the RNase-free Spin Column CR2 in a 2-mL RNase-free collection tube (not provided) without wetting the rim. Close the cap and centrifuge at 8000 rpm (approximately 6000 × g) for 1 min. Discard the filtrate; place the RNase-free Spin Column CR2 back in the collection tube.

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

8. Repeat step 7.
9. Carefully open the RNase-free Spin Column CR2, and add 500 µL of Buffer GBR (ensure ethanol [96%–100%] has been added to the Buffer GBR before use) without wetting the rim. Close the cap and centrifuge at 8000 rpm (approximately 6000 × g) for 1 min. Discard the filtrate and place the RNase-free Spin Column CR2 back in the collection tube.
10. Carefully open the RNase-free Spin Column CR2, and add 500 µL of Buffer WB (ensure ethanol [96%–100%] has been added to the Buffer WB before use) without wetting the rim. Close the cap and centrifuge at 8000 rpm (approximately 6000 × g) for 1 min. Discard the filtrate and place the RNase-free Spin Column CR2 back in the collection tube.
11. Centrifuge at full speed (12,000 rpm; approximately 13,400 × g) for 3 min to dry the membrane completely.

Note: Ethanol carryover into the eluate may cause problems in downstream applications.

12. Recommended: Place the RNase-free Spin Column CR2 back into the same 2-mL collection tube, open the lid, and incubate at room temperature (15–25 °C) for 3 min to dry the membrane completely.

13. Place the RNase-free Spin Column CR2 in a clean 1.5-mL RNase-free Microcentrifuge Tube, and discard the old collection tube with the filtrate. Carefully open the lid of the RNase-free Spin Column CR2, and apply 60 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 8000 rpm (approximately 6000 × g) for 1 min.

Note: Ensure that the elution buffer (RNase-free ddH_2O) 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. Adjust the volume of elution buffer according to the specific requirements of the experiment. Incubate at room temperature (15–25 °C) for 5 min to increase the RNA yield after RNase-free ddH_2O is added to the RNase-free Spin Column CR2.