



PURO Virus DNA/RNA Kit

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



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PURO Virus DNA/RNA Kit

Cat. no. SC0201

Kit Contents

Contents	SC0201 (50 preps)
Buffer GB	15 ml
Buffer GD	13 ml
Buffer PW	15 ml
RNase-Free ddH ₂ O	15 ml
Proteinase K	1 ml
Carrier RNA	310 µg
RNase-Free ddH ₂ O	1 ml
RNase-Free Spin Columns CR2	50
RNase-Free Collection Tubes 2 ml	50
RNase-Free Centrifuge Tubes 1.5 ml	50
Handbook	1

Storage

PURO Virus DNA/RNA Kit should be stored dry at room temperature (15-25°C) and is stable for 12 months. For longer storage, the kit can be stored at 2-8°C. If there is precipitates formed under 2-8°C, please place the kit under room temperature. If necessary, warming in 37°C water bath for 10 min to dissolve precipitates. Carrier RNA can only be dissolved in RNase-Free ddH₂O; dissolved Carrier RNA should be stored at -20°C.

Introduction

The PURO 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. PURO Virus DNA/RNA Kit uses silica membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. Viral DNA/RNA purified with PURO 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, southern blot and so on.

Important notes

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

Preparation of Carrier RNA solutions

- Add 310 μ l RNase-Free ddH₂O to the tube containing 310 μ g lyophilized Carrier RNA to obtain a solution of 1 μ g/ μ l. 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 3 times.
- Carrier RNA cannot be dissolved in Buffer GB directly. It must first be dissolved in RNase-Free ddH₂O and then added to Buffer GB.
- Carrier RNA working solution: Calculate the volume of Buffer GB/Carrier RNA mix required per batch of samples by selecting the number of samples to be simultaneously processed from table 1. For larger numbers of samples, volumes can be

calculated using the following sample calculation

$$n \times 0.22 \text{ ml} = y \text{ ml}$$

$$y \text{ ml} \times 28 \mu\text{l/ml} = z \mu\text{l}$$

n = number of samples to be processed simultaneously

y = calculated volume of Buffer GB

z = volume of Carrier RNA/RNase-Free ddH₂O to add to Buffer GB

Table 1 Volumes of Buffer GB and Carrier RNA/RNase-Free ddH₂O Mix required for the Procedure

Number of Samples	Vol. Buffer GB (ml)	Vol. Carrier RNA/RNase-Free ddH₂O (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 GB with Carrier RNA solution upside down. Do not vortex to avoid bubbling.

Protocol

Please add ethanol (96-100%) to Buffer PW before use, the volume as described on the bottle.

1. Pipet 20 μ l Proteinase K into a clean 1.5 ml centrifuge tube (not provided).
2. Add 200 μ l of plasma or serum into the centrifuge tube (Equilibrate the samples to room temperature.).

Note: If the sample volume is less than 200 μ l, add the appropriate volume of 0.9% sodium chloride solution to bring the volume of protease and sample up to a total of 220 μ l.

3. Add 200 μ l Buffer GB (containing 28 μ g/ml of Carrier RNA). Close the cap and mix by pulse-vortex for 15 s.

Note: In order to ensure efficient lysis, it is essential that the sample and Buffer GB 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 μ l of ethanol (96-100%) to the sample (Precipitates may be visible after addition of ethanol), close the cap and mix thoroughly by pulse-vortex for 15 s. Incubate the lysate with the ethanol for 5 min at room temperature (15-25°C).
Note: Cool ethanol (96-100%) on ice before use if the room temperature is more than 25°C.
6. Briefly centrifuge the 1.5 ml centrifuge tube to remove drops from the inside of the lid.
7. Carefully transfer the lysate, including any precipitates that may have formed onto the RNase-Free Spin Columns CR2 in a 2ml RNase-Free Collection Tube without wetting the rim. Close the cap and centrifuge at 8,000 rpm (\sim 6,000 \times 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 the RNase-Free Spin Columns CR2 after centrifugation, centrifuge again at higher speed until the spin column is empty.
8. Carefully open the RNase-Free Spin Columns CR2, and add 500 μ l of Buffer GD (Ensure that ethanol (96-100%) has been added into Buffer GD before use) without wetting the rim. Close the cap and centrifuge at 8,000 rpm (\sim 6,000 \times 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 μ l of Buffer PW (Ensure that ethanol (96-100%) has been added into Buffer PW before use) without wetting the rim. Close the cap, let it stand still for 2 min and centrifuge at 8,000 rpm (\sim 6,000 \times 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 μ l of ethanol (96-100%) without wetting the rim. Close the cap and centrifuge at 8,000 rpm ($\sim 6,000 \times g$) for 1 min. Discard the filtrate.

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

12. Place the RNase-Free Spin Column CR2 in the same collection tube. Centrifuge at full speed 12,000 rpm ($\sim 13,400 \times g$) for 3 min to dry the membrane completely.
13. Place the RNase-Free Spin Column CR2 in a clean 1.5 ml RNase-Free Centrifuge Tube, carefully open the lid of the spin column, incubate at room temperature for 3 min to dry the membrane completely. Add 20-150 μ l of RNase-Free ddH₂O 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 ($\sim 13,400 \times g$) for 1 min.

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