Version: FD161115

FinePure Virus RNA Kit

(Spin Column)

Cat. no. FR501

Kit Contents

Contents	FR501 (50 preps)
Buffer DLT	15 ml
Buffer DW1	13 ml
Buffer DW2	12 ml
RNase-Free ddH_2O	15 ml
Proteinase K	1 ml
Carrier RNA	310 μg
FinePure Mini Spin Columns	50
Collection Tubes(2 ml)	50
1.5 ml RNase-Free Microcentrifuge Tubes	50
Handbook	1

Storage

FinePure Virus RNA Kit should be stored dry at room temperature (15-25°C) and is stable for 12 months. Check Buffer DLT for precipitate, and if necessary incubate at 60°C until the precipitate is dissolved. Carrier RNA should be dissolved in RNase-Free ddH_2O. Unused portions of carrier RNA dissolved in RNase-Free ddH_2O should be frozen in aliquots at -20°C. Do not freeze-thaw the aliquots of carrier RNA more than 3 times.

Introduction

FinePure Virus RNA Kit provides the fastest and easiest way to purify viral RNA for reliable use in amplification technologies. It is suitable for purification of viral RNA from plasma, serum, and other cell-free body fluids and the procedure is optimized for use with 200 µl samples. Samples may be fresh or frozen, but if frozen, should not be thawed more than once. Repeated freeze-thawing of plasma samples will lead to reduced viral titers and should be avoided for optimal sensitivity. Cryoprecipitates accumulate when samples are subjected to repeated freeze-thaw cycles. This may lead to clogging of the FinePure membrane when using the vacuum protocol.

Carrier RNA, added to Buffer DLT, improves the binding of viral RNA to the FinePure membrane especially in the case of low-titer samples, and limits possible degradation of the viral RNA due to any residual RNase activity. High-quality RNA is eluted in a special RNase-Free buffer, ready for direct use or safe storage.

Important notes

1. All protocol steps should be carried out at room temperature (15-25°C).
2. Equilibrate samples to room temperature(15-25°C).
3. Equilibrate RNase-Free ddH_2O to room temperature(15-25°C).

Preparation of Carrier RNA solutions

- Add 310 µl RNase-Free ddH_2O to the tube containing 310 µg 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 3 times.
- Carrier RNA cannot be dissolved in Buffer DLT directly. It must first be dissolved in RNase-Free ddH_2O and then added to Buffer DLT.

Carrier RNA working solution: Calculate the volume of Buffer DLT/Carrier RNA mix required per batch of samples by selecting the number of samples to be simultaneously processed from table 1.

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

No. samples	Vol. Buffer DLT (ml)	Vol. carrier RNA-RNase-Free ddH_2O (µl)
n	220 µl × n	4 µl × n

Note：The sample-preparation procedure is optimized for 4µl of carrier RNA per sample. Gently mix by inverting the tube 10 times. To avoid foaming, do not vortex.

Protocol

Please add ethanol (96-100%) to Buffer DW1 and DW2 before use, the volume as described on the bottle.

1. Pipet 20 µl Proteinase K into a clean 1.5 ml Microcentrifuge Tubes (not provided).
2. Add 200 µl of plasma, serum, urine, cell-culture supernatant, or cell-free body fluid into the Microcentrifuge Tubes (Equilibrate the samples to room temperature).

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

3. Add 200 µl Carrier RNA working solution (mixture of Buffer DLT and Carrier RNA solution, please refer to table 1). Close the cap and mix by pulse-vortex for 15 s.

Note: To ensure efficient lysis, it is essential that the sample is mixed thoroughly with Buffer DLT to yield a homogeneous solution. Frozen samples that have only been thawed once can also be used.

4. Incubate at 72°C for 10 min in a heating block. Briefly centrifuge the 1.5 ml tube to remove drops from the inside of the lid.
5. Add 100 µl of isopropanol to the sample (Precipitates may be visible after addition of isopropanol or ethanol), close the cap and mix thoroughly by pulse-vortex for 15 s.

Note: We recommend the use of 100 µl isopropanol. In the absence of isopropanol, it can be replaced by 250 µl ethanol (96-100%).

6. Briefly centrifuge the 1.5 ml centrifuge tube to remove drops from the inside of the lid.
7. Carefully transfer all of the solution from step 6 to the FinePure Mini Spin Column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 12,000 rpm 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 FinePure Mini Spin Column after centrifugation, centrifuge again at higher speed until the spin column is empty.

8. Carefully open the FinePure Mini Spin Column, and add 500 µl of Buffer DW1 (**Ensure that ethanol (96-100%) has been added before use**) without wetting the rim. Close the cap and centrifuge at 12,000 rpm for 1 min. Discard the filtrate and place the spin column in the same collection tube.
9. Carefully open the FinePure Mini Spin Column, and add 450 µl of Buffer DW2 (**Ensure that ethanol (96-100%) has been added before use**) without wetting the rim. Close the cap, let it stand still for 2 min and centrifuge at 12,000 rpm for 1 min. Discard the filtrate and place the spin column in the same collection tube.
10. Repeat step 9.
11. Place the FinePure Mini Spin Column in the same collection tube. Centrifuge at full speed 12,000 rpm for 3 min to dry the membrane completely.
12. Place the FinePure Mini Spin Column in a clean 1.5 ml RNase-Free Microcentrifuge Tube. Add 40 µl of RNase-Free ddH_2O to the center of the membrane. Close the lid and incubate at room temperature (15-25°C) for 2 min. Centrifuge at 12,000 rpm for 2 min.

Note: Equilibrate RNase-Free ddH_2O to room temperature. RNase-Free ddH_2O should be dispensed onto the center of the membrane for complete elution of bound RNA.