Ver.23121

ODP318

Virus DNA/RNA Kit

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

Origin®

ISO 13485:2016 ISO 9001:2015

Virus DNA/RNA Kit

Cat No. ODP318

Kit contents

Contents	50 preps
Buffer GB	15mL
Buffer GD	13mL
Buffer PW	15mL
RNase-free ddH_2O	15mL
Proteinase K	1mL
Carrier RNA	250μL × 2
RNase-free Spin Columns CR3	50
Collection Tube (2mL)	50
Handbook	1

Storage

Virus DNA/RNA Kit can 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 precipitates are formed when stored at 2-8°C, place the kit under room temperature. If necessary, warming in 37°C water bath for 10 minutes to dissolve precipitates. Carrier RNA should be stored at -20°C.

Introduction

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. Virus DNA/RNA kit uses silica membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. Viral DNA/RNA extraction can be completed in typically 10–30 minutes with DNA recoveries of 60–90%. Viral DNA/RNA purified with Virus DNA/RNA kit is immediately ready for use. Phenol extraction is 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 1.5mL are used in step 13. Others are not supplied.

Materials required but not provided

1.5mL microcentrifuge tubes, ethanol (96-100%)

Protocol

Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

1. Pipette 20µL Proteinase K into a clean 1.5mL centrifuge tube (not provided).
2. Add 200µL of plasma/serum/ lymph into the centrifuge tube (Equilibrate the samples to room temperature.).
   Note: If the sample volume is less than 200µL, add the appropriate volume of 0.9% sodium chloride solution to bring the volume to a total of 220µL.
3. Incubate at 56°C for 15 minutes in a heating block. Briefly centrifuge the 1.5mL tube to remove drops from the inside of the lid.
4. Add 200µL Buffer GB to the sample, mix thoroughly by vortexing, and incubate at 70°C for 10 minutes to yield a homogeneous solution. Briefly centrifuge the 1.5mL microcentrifuge tube to remove drops from the inside of the lid.
5. Add 10µL carrier RNA and mix the contents by vortexing for 10 seconds.
   Note: Add carrier RNA after the mixture cools down to room temperature
6. Add 250µL 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 seconds. Incubate the lysate with the ethanol for 5 minutes at room temperature (15-25°C).
   Note: Cool ethanol (96-100%) on ice before use if the room temperature is more than 25°C.
7. Briefly centrifuge the 1.5mL centrifuge tube to remove drops from the inside of the lid.
8. Carefully transfer the lysate, including any precipitates that may have formed onto the RNase-free Spin Columns CR3 in a 2mL RNase-free Collection Tube without wetting the rim. Close the cap and centrifuge at 8,000 rpm (~6,000 × g) for 1 minute. 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 CR3 after centrifugation, centrifuge again at higher speed until the spin column is empty.
9. Carefully open the RNase-free Spin Columns CR3, and add 500µL of Buffer GD (Ensure that ethanol (96-100%) has been added before use) without wetting the rim. Close the cap and centrifuge at 8,000 rpm (~6,000 × g) for 1 minute. Discard the filtrate and place the spin column in the same collection tube.

10. Carefully open the RNase-free Spin Columns CR3, and add 700µL of Buffer PW (Ensure that ethanol (96-100%) has been added before use) without wetting the rim. Close the cap, let it stand still for 2 minutes and centrifuge at 8,000 rpm (~6,000 × g) for 1 minute. Discard the filtrate and place the spin column in the same collection tube.
11. Repeat step 10.
12. Place the RNase-free Spin Column CR3 in the same collection tube. Centrifuge at full speed 12,000 rpm (~13,400 × g) for 3 minutes to dry the membrane completely.
13. Place the RNase-free Spin Column CR3 in a clean 1.5mL RNase-free Centrifuge Tube, carefully open the lid of the spin column, incubate at room temperature for 3 minutes to dry the membrane completely. Add 20-150µL of RNase-free ddH_2O to the center of the membrane. Close the lid and incubate at room temperature (15-25°C) for 5 minutes. Centrifuge at 12,000rpm (~13,400 × g) for 1 minute.

    Note: Ensure that the RNase-free ddH_2O is equilibrated to room temperature (15- 25°C). If elution is done in small volumes (<50µL), the RNase-free ddH_2O should be dispensed onto the center of the membrane for complete elution of bound RNA and DNA