Viral RNA+DNA Preparation Kit

Cat#: orb653796 (Product Manual)

Description:

Viral RNA+DNA Preparation Kit is designed for rapid and effective isolation of RNA and DNA from a variety of pathogen organisms such as virus or bacteria. Samples can be fresh or frozen plasma/blood (treated with anticoagulants excepted heparin), serum, other cell-free body fluids or pathogen-infected tissue. The kit allows high yield isolation of viral RNA/DNA from nasal or throat swabs.

The Kit is specifically designed to isolate high-quality nucleic acids using low elution volumes and allowing sensitive downstream ana-lysis including quantitative PCR and RT-PCR. The purified RNA/DNA is free of proteins and nucleases. Viral RNA+DNA Preparation Kit uses lysis buffer including chaotropic salts to inactivate RNases/DNases and advanced silica-gel membrane technology for fast purification of intact RNA/DNA. The preparation procedure is optimized to give reproducible results within 30 min.

Content:

Component	50 Prep Kit	5 x 50 Prep Kit
PBS Buffer	6 ml	30 ml
Ethanol (96-99 %)	fill up with 15 ml Ethanol	fill up with 75 ml Ethanol
Lysis Buffer	15 ml	75 ml
Washing Buffer A	15 ml (add 15 ml ethanol before use)	75 ml (add 75 ml ethanol before use)
Washing Buffer B	6.2 ml (add 25 ml ethanol before use)	31.2 ml (add 125 ml ethanol before use)
Elution Buffer	3 ml	15 ml
Spin Columns and Collection Tubes	50 spin columns and collection tubes	5 x 50 spin columns and collection tubes

Additional Materials Required:

96-99 % Ethanol

1.5 ml Tubes

Preparation procedure:

The DNA purification follows a cell lysis, RNA/DNA binding, washing and eluting procedure. Before starting, add Ethanol (96-99 %, not included in the kit) to Washing Buffer A and B as indicated on data sheet and bottles. Please note that the Ethanol concentration of a Washing Buffer may decrease during long term storage resulting in a drop-down of the final DNA yield.

The provided Lysis Buffer contains carrier molecules to enhance binding of RNA/DNA on the column membrane.

1a Preparation from plasma, serum, urine, cell-culture super-nantant, cell-free fluid or virus infected tissue

- Transfer 100 µl plasma, serum, urine, cell-culture supernatant, cell-free fluid or virus infected tissue into a 1.5 ml microtube.
- Note: Adjust lower sample volumes with PBS Buffer to 100 µl. Samples of larger volumes (up to 200 µl) can easily be scaled up but may require larger tubes for the lysis procedure.
- Add 250 µl (2.5 amounts of sample volume) of Lysis Buffer.
- Vortex for 15 sec.
- Incubate at room temperature (20-25 °C) for 10 min.
- Add 250 µl (2 amounts of sample volume) Ethanol (96-99 %) and mix well by gently vortexing.

1b Preparation from nasal or throat swabs

- Transfer 100 µl PBS Buffer into a 1.5 ml microtube.
- Add 250 µl of Lysis Buffer.
- Cut off the cotton tip with the collected nasal or throat cells and place it in the micro tube.
- Close the tube and vortex for 15 sec.
- Incubate at room temperature (20-25 °C) for 10 min.
- Remove the cotton tip and squeeze it out at the rim of the tube.
- Add 250 µl Ethanol (96-99 %) and mix well by gently vortexing

2 Column Loading

- Place a Spin Column into a provided 2 ml collection tube.

- Spin down the Lysate-Ethanol mixture and transfer the solution into the Spin Column.
- Close the cap and centrifuge the Spin Column at 13,000 g for 1 min.
- Discard the flow-through in the collection tube and place the column back in the same tube.
- Note: The maximum volume of the column reservoir is 800 µl. For larger sample volumes discard the flow-through in-between and load the spin column again.

3 Column Washing

- Add 500 µl Washing Buffer A (Ethanol added) to the Spin Column and centrifuge at 13,000 g for 1 min.
- Discard the flow-through in the collection tube and place the

Spin Column back in the same tube.

- Add 500 µl Washing Buffer B (Ethanol added) to the Spin Column and centrifuge at 13,000 g for 1 min.
- Note: Before using Washing Buffer B for the first time add eth-anol as indicated on the bottle.
- Discard the flow-through in the collection tube and place the Spin Column back in the same tube.
- Centrifuge at 13,000 g for 1 min.
- Note: It is important to dry the membrane since residual eth-anol may interfere with downstream reactions.

4 Elution

- Place the Spin Column into a new 1.5 ml microtube (not provided).
- Add 40-50 µl Elution Buffer directly onto the membrane of the spin column.
- Note: Avoid touching membrane with the pipet tip.
- Incubate at room temperature for 1 min.
- Centrifuge at 13,000 g for 1 min.
- Use 2-5 µl of the eluted RNA and/or DNA as template in PCR or RT-PCR assays or for further down-stream applications. The eluted RNA/DNA can be stored at -70 °C.