



Viral RNA+DNA Preparation Kit - Column Kit

Spin column based RNA+DNA purification from blood, serum, tissue, cell culture or swabs

Cat. No.	Amount
PP-235S	50 preparations
PP-235L	250 preparations

For general laboratory use.

Shipping: shipped at ambient temperature

Storage Conditions: store at ambient temperature

Shelf Life: 12 months

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 except 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 analysis 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 achieve reliable results within 30 min.

Content:

Component	PP-235S 50 Prep Kit	PP-235L 5 x 50 Prep Kit
Lysis Buffer	15 ml	75 ml
Ethanol (96-99 %)	fill up with 15 ml Ethanol	fill up with 75 ml Ethanol
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 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



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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 nasal or throat swabs

- Transfer 250 µl of **Lysis Buffer** into a microcentrifuge tube.
- 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.

1b Preparation from plasma, serum, urine, cell-culture supernatant, 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: 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 (or 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 (or 2.5 amounts of sample volume) **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 rpm 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 rpm 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 rpm for 1 min.

- Note: Before using Washing Buffer B for the first time add ethanol 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 rpm for 1 min.
- Note: It is important to dry the membrane since residual ethanol 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 rpm 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.