KIAGENE FANAVAR

DNAVIRO
Viral DNA/RNA Isolation Kit

Cat. No:

FPKT004.0025
FPKT004.0050
FPKT004.0100

Contents:

Components	25prep	50prep	100prep
VDR1 buffer	8 ml	16 ml	36 ml
VDR2 buffer	10 ml	20 ml	40 ml
VDR3 buffer	2.5 ml	5 ml	10 ml
ER buffer	1 ml	2 ml	4 ml
Proteinase K (20mg/ml)	250 µl	500 µl	1 ml
RNA Carrier (1µg/µl)	150 µl	300 µl	600 µl
Column and collection tubes	25 each	50 each	100 each

Kit storage:

⚠ *This kit should be stored at room temperature.*

⚠ ***Proteinase K*** *and dissolved* ***RNA carrier*** *should be stored at -20 °C*

If properly stored, all kit components are stable until the expiration date printed on the label.

Additional Equipment and Reagent required

- Absolute ethanol
- Standard tabletop microcentrifuge capable of 13,000 x *g* centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile
- VTM or PBS

Application

Viral DNA/RNA extraction kit is designed for rapid and effective isolation of viral DNA/RNA from plasma, serum, cell-free body fluid and cell culture supernatant.

Samples can be fresh or frozen plasma/blood (treated with anticoagulants except heparin), serum, other cell-free body fluids.

The kit allows high yield isolation of viral DNA/RNA 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 DNA/RNA is free of proteins and nucleases. Viral DNA/RNA extraction kit uses lysis buffer including chaotropic salts to inactivate RNases/DNases and advanced silica-gel membrane technology for fast purification of intact DNA and RNA. The procedure is optimized to achieve reliable results within **30 min.**

Handling Requirements and Safety Information

⚠ *All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved.*

⚠ *VDR1 Buffer and VDR2 Buffer contain guanidinium hydrochloride which is an irritant.*

⚠ *Do not allow VDR1 Buffer and VDR2 Buffer to touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water.*

⚠ *If you spill the reagent, dilute the spill with water before wiping it up.*

⚠ *Do not use any modified ethanol.*

⚠ *Do not pool reagents from different lot numbers.*

⚠ *Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions.*

⚠ *After first opening store all bottles in an upright position.*

⚠ *Do not allow the VDR1 Buffer and VDR2 Buffer to mix with sodium hypochlorite found in commercial bleach solutions. This mixture can produce a highly toxic gas.*

⚠ Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.

⚠ Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles.

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preparation procedure:

Working Solution Preparation

The DNA/RNA purification follows a cell lysis, DNA/RNA binding, washing and eluting procedure. Before starting, add Ethanol (Absolute ethanol) to VDR2 and VDR3 Buffers:

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⚠ **Add 2.5 ml absolute ethanol to VDR2 Bottle**

⚠ **Add 10 ml absolute ethanol to VDR3 Bottle**

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⚠ **Add 5 ml absolute ethanol to VDR2 Bottle**

⚠ **Add 20 ml absolute ethanol to VDR3 Bottle**

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⚠ **Add 10 ml absolute ethanol to VDR2 Bottle**

⚠ **Add 40 ml absolute ethanol to VDR3 Bottle**

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

⚠ If you want to use the whole kit (100 purifications) at the same time, **add RNA Carrier and Proteinase K completely** to VDR1 Buffer bottle and **add 366 µl of VDR1 Buffer to each sample instead of 350 µl.**

⚠ before starting Incubate the **ER buffer at 55 to 65 ° C** until the end of the protocol to obtain the maximum yields.

Protocol

1. Sample preparation

1a)

nasal or throat swabs without Viral Transform Media:
Transfer 250 µl of VTM or PBS into a 1.5 ml microcentrifuge tube. Cut off the cotton tip with the collected nasal or throat cells and place it in the microtube. 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. Transfer 150 µl of VTM or PBS containing the virus into a 1.5 ml microcentrifuge tube. **Add 350 µl of VDR1 Buffer**. Vortex for 15 sec.

1b)

Preparation from plasma, serum, urine, cell-culture supernatant, cell-free fluid or virus infected tissue and nasal or throat swabs in Viral Transform Media :
Transfer 150 µl plasma, serum, urine, cell-culture supernatant, VTM or PBS containing the virus, cell-free fluid or virus infected tissue into a 1.5 ml microcentrifuge tube. **Add 350 µl of VDR1 Buffer**. Vortex for 15 sec.

2. To each sample add **6 µl RNA Carrier and 10 µl Proteinase K.** incubate the mixture at **65°C for 15 min**.

3. Add 500 µl Absolute ethanol to each sample.

4. Insert one High Pure Filter Tube in a Collection Tube. Pipette entire sample into the upper of the Filter Tube. **Centrifuge the tube assembly 4000 g for 5 min.**

5. Discard the flow through liquid .**Add 500 µl VDR2** to the upper of the Filter Tube and **centrifuge 8000 g, 1 min.**

6. Discard the flow through liquid. **Add 500 µl VDR3** to the upper of the Filter Tube. **Centrifuge 8000 g, 1 min** and discard the flow through.

7. Leave the tube assembly in the centrifuge and spin it for **3 min at maximum speed** (approximately 13,000 g) to remove any residual Wash Buffer.

8. Discard the Collection Tube and insert the Filter Tube into a clean, sterile 1.5 ml microcentrifuge tube.

9. Add 50 µl ER Buffer to the upper of the Filter Tube. Centrifuge the tube assembly for **8000 g, 1 min**.

⚠ to obtain the maximum yields you can add the RNA solution to the top of the filter tube and repeat Centrifuge the tube assembly for **8000 g, 1 min**.

The **microcentrifuge** tube now contains the eluted viral DNA/RNA. Either use the eluted RNA directly in RT.PCR or store the eluted RNA at −70°C for later analysis.