KIAGENE FANAVAR

RENAVIRO
Viral RNA extraction kit
Cat. No: FPKT033.0025
Cat. No: FPKT033.0050
Cat. No: FPKT033.0100
Contents:

Components	25prep	50prep	100prep
LR buffer	8 ml	16 ml	36 ml
WR1 buffer	10 ml	20 ml	40 ml
WR2 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 RNA extraction kit is designed for rapid and effective isolation of viral RNA from plasma, serum, cell-free body fluid and cell culture supernatant.
The procedure is optimized to achieve reliable results within **30 min**.

Description:

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 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 RNA is free of proteins and nucleases. Viral RNA extraction kit uses lysis buffer including chaotropic salts to inactivate RNases/DNases and advanced silica-gel membrane technology for fast purification of intact RNA.

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.*

⚠ *LR Buffer and WR1 Buffer contain guanidinium hydrochloride which is an irritant.*

⚠ *Do not allow LR Buffer and WR1 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 LR Buffer and WR1 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 RNA purification follows a cell lysis, RNA binding, washing and eluting procedure. Before starting, add Ethanol (Absolute ethanol) to Washing Buffer:

Cat. No: FPKT033.0025 25prep

⚠ **Add 2.5 ml absolute ethanol to WR1 Bottle**

⚠ **Add 10 ml absolute ethanol to WR2 Bottle**

Cat. No: FPKT033.0050 50prep

⚠ **Add 5 ml absolute ethanol to WR1 Bottle**

⚠ **Add 20 ml absolute ethanol to WR2 Bottle**

Cat. No: FPKT033.0100 100prep

⚠ **Add 10 ml absolute ethanol to WR1 Bottle**

⚠ **Add 40 ml absolute ethanol to WR2 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 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 LR buffer bottle and **add 366 µl of LR 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 <u>without</u> 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 LR Buffer**. Vortex for 15 sec.

1b)

Preparation from plasma, serum, urine, cell-culture supernantant, 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 LR Buffer**. Vortex for 15 sec.

2. To each sample add **6 µl RNA Carrier and 10 µl Proteinase K.** incubate the mixture at **58°C for 10 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 30 sec at 10000 rpm**.

5. Discard the flow through liquid .**Add 500 µl WR1** to the upper of the Filter Tube and **centrifuge 30 sec at 10000 rpm.**

6. Discard the flow through liquid. **Add 500 µl WR2** to the upper of the Filter Tube. **Centrifuge 30 sec at 10000 rpm** and discard the flow through.

7. Leave the tube assembly in the centrifuge and spin it for **3 min at maximum speed** (approximately 14,000 rpm) 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 **1 min at 8,000 rpm**.

⚠ 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 **1 min at 8,000 rpm**.

The **microcentrifuge** tube now contains the eluted viral RNA. Either use the eluted RNA directly in RT.PCR or store the eluted RNA at –20°C for later analysis.

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Troubleshooting

Observation	Possible cause	Recommendation
Low nucleic acid yield or purity	Kit stored under non-optimal conditions	Store kit at +15 to +25°C at all times upon arrival.
	Buffers or other reagents were exposed to conditions that reduced their effectiveness.	Store all buffers at +15 to +25°C.
		Close all reagent bottles tightly after each use to preserve pH, stability, and freedom from contamination.
		store the ***Proteinase K*** *and dissolved* ***RNA carrier*** at -15 to -25°C.
	Ethanol not added to Wash Buffer	Add absolute ethanol to the buffers before using.
		After adding ethanol, mix the buffers well and store at +15 to +25°C.
		Always mark Wash Buffer vial to indicate whether ethanol has been added or not.
	Reagents and samples are not completely mixed.	Always mix the sample tube well after addition of each reagent.
Poor elution of nucleic acids with water	Water has the wrong pH.	If you use your own water or buffer to elute nucleic acids from Filter Tube, be sure it has the same pH as the Elution Buffer supplied in the kit.
Absorbance ($A_{260\ nm}$) reading of product too high	Glass fibers, which might coelute with nucleic acid, scatter light.	• Remove High Pure Filter Tube from tube containing eluted sample and spin sample for 1 min at maximum speed. • Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube.
Low RNA yield	High levels of RNase activity.	Be careful to create an RNase-free working environment
		Process starting material immediately or store it at -80°C until it can be processed.
		Use eluted RNA directly in downstream procedures or store it immediately at -80°C.