FG-82050 50 Prep FastGene® RNA Viral Kit

FG-82300 300 Prep FastGene® RNA Viral Kit

Kit Contents

Components		Quantity		Storage
Cat. No.		FG-82050	FG-82300	
Buffer ViL	*(Lysis Buffer)*	30 ml	180 ml	
Buffer ViB (concentrate)*	*(Binding Buffer)*	8 ml	48 ml	Room
Buffer ViW1 (concentrate)*	*(Wash Buffer 1)*	13 ml	104 ml	temperature
Buffer ViW2 (concentrate)*	*(Wash Buffer 2)*	6 ml	36 ml	(15 ~ 25°C)
Nuclease free Water		15 ml	90 ml	
FastGene® Column Vi (with collection tube)		50	300	
1.5 ml microcentrifuge tube		50	300	

-*Before using for the first time, add absolute ethanol into buffer ViB, ViW1 and ViW2 as indicated on the bottle.

Specifications

Specifications	FastGene® RVK
Type	Spin column
Maximum amount of starting samples	300 µl / prep
Preparation time	20 minutes
Maximum loading volume	800 µl
Minimum elution volume	30 µl

Quality Control

FastGene® RNA viral kits are manufactured in strictly clean room condition. Cleanness is monitored regularly. Each lot must pass the quality control and only kits which pass these tests are delivered.

Storage Conditions

FastGene® RNA viral kit should be stored at room temperature (15 ~ 25°C). **Storage at higher temperatures should be avoided**. The expiry date is written at the box label.

Precautions

The buffers included in FastGene® RNA Viral Kit contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector. Follow standard safety precautions. In case of contact, wash immediately with plenty of water and seek medical advice. Buffer ViL, ViB, and ViW2 contain chaotropic salts, which can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

Preventing RNase Contamination

RNase can be introduced accidentally during RNA preparation. Always wear disposable gloves as skin often contains bacteria that can be a source of RNase. Use sterile, disposable plastic- wares and pipettes reserved for RNA work to prevent cross-contamination with RNase on shared equipment.

Product description

The FastGene® RNA viral kit is intended for molecular biology applications. This product is NOT intended for the diagnosis, prevention or treatment of a disease. FastGene® RNA viral kit provides a suitable method for isolation of RNA from plasma, serum, swab, urine, cell-free fluid, cell-culture supernatant and virus-infected samples.

FastGene® RNA viral kit procedures employed the silica membrane technology for the fastest and the most convenient of high purity RNA isolation, instead of conventional alcohol precipitation or phenol/chloroform extraction.

FastGene® RNA viral kit buffer system provides effective binding condition of RNA silica membrane through mix with lysis and binding buffers. By using two different wash buffers all impurities, which can be left on the membrane are washed away. At last, pure RNA is eluted by nuclease-free water. From sample to isolated RNA you only need 15 minutes and the eluate is suitable for PCR, RT-PCR, or any downstream application without further manipulation. FastGene® RNA viral kit procedure should be performed at room temperature. The eluate should be treated with care because RNA is very sensitive to contaminants, such as RNases, often found on general labware and dust. To ensure RNA-stability, storage at 4°C is recommended for immediate analysis or freezing at -70°C for long-term storage

Protocol for FastGene® RNA viral kit

1.Transfer up to 300 µl of sample (swab-storage media, cell-free fluid, cell-culture supernatant, plasma, serum, urine) in a 1.5 ml microcentrifuge tube.

2.Add 500 µl of buffer ViL (Lysis Buffer) to the tube and lyse the sample by vortexing for 15 seconds.
Check buffer ViL for precipitation. Precipitate can be solved by incubation at 37°C or above.
The volume of buffer ViL can be adjusted in proportion to the volume of sample.
For proper lysis, the complete mix of sample and buffer ViL is essential.

3.Incubate the lysate for 10 minutes at room temperature (15 – 25°C).
After this step, briefly centrifuge the tube to remove drops from the inside of the lid.

4.Add 700 µl of buffer ViB (Binding Buffer) to the lysate and mix thoroughly by inverting or vortexing.
The volume of buffer ViB can be adjusted in proportion to the volume of lysate.

Do not centrifuge!

5.Transfer up to 750 µl of the mixture to the FastGene® Column Vi.

6.Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.
Discard the pass-through and reinsert the FastGene® Column Vi back into the same tube.

7.For larger volumes repeat step 5 ~ 6 with the remainder of the sample.
Discard the pass-through and reinsert the FastGene® Column Vi back into the same tube.

8.Add 500 µl of buffer ViW1 (Wash Buffer) to the FastGene® Column Vi.

9.Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.
Discard the pass-through and reinsert the FastGene® Column Vi back into the same tube.

10.Add 500 µl of buffer ViW2 (Wash Buffer 2) to the FastGene® Column Vi.

11.Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.
Discard the pass-through and reinsert the FastGene® Column Vi back into the same tube.

12.Centrifuge at ≥ 10,000 x g for an additional 1 minute at room temperature to remove residual wash buffer.
Transfer the FastGene® Column Vi to a new 1.5 ml microcentrifuge tube (provided). Residual Buffer ViW2 in the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, resulting in flow through, containing Buffer ViW2, contacting the FastGene® column. It is important to dry the membrane since residual ethanol may interfere with downstream reactions.

13.Add 30 ~ 50 µl of nuclease-free water (provided) to the center of the membrane in the FastGene® Column Vi. Incubate at room temperature for 1 minute.

14.Centrifuge at ≥ 10,000 x g for 1 minute at room temperature.
Purified RNA can be stored at 4°C for immediate analysis or can be stored at -70°C for long term storage. Viral RNA is stable for up to one year when stored under these conditions.

Troubleshooting Guide

Problem	Possible cause	Suggested solution
Low yield	Poor quality of starting material	Repeated freezing and thawing should be avoided. Always use fresh samples or samples thawed only once
	Low concentration of virus in the sample	Use more sample. Concentrate the sample volume to 300 µl using a micro concentrator (ultrafiltration)
	Sample not homogenized completely	Be sure to incubate for 10 minutes at room temperature after lysis. For proper lysis, please mix sample and buffer ViL thoroughly
	Incorrect elution conditions	Add nuclease-free water to the center of the mini spin column membrane and perform incubation for 1 minute before centrifugation.
	Precipitation of buffer ViL	Storage at low temperature may lead to precipitates in buffer ViL. Precipitation can be avoided by incubating the buffer at 37°C or higher (max. at 80°C) until it disappears.
	Degradation of RNA	RNase can be introduced during use. Be certain not to introduce any RNases during the procedure or later handling. Keep tubes closed whenever possible during the preparation
	Buffer ViW1 and ViW2 used in the wrong order	Ensure that buffer ViW1 and ViW2 are used in the correct order, shown in the protocol. If used in the wrong order, perform the last washing step with ViW2.
Eluate does not perform well in downstream application	Residual ethanol remains in eluate	Centrifuge again to remove ethanol, which is included in buffer ViW2 from mini spin column membrane. (step 12)
	Buffer ViW1 and ViW2 used in the wrong order	Ensure that buffer ViW1 and ViW2 are used in the correct order, shown in the protocol. If used in the wrong order, perform the last washing step with ViW2.