

vivantis
Nucleic Acid Extraction Kit HandBook



GF-1

**VIRAL NUCLEIC ACID
EXTRACTION USER GUIDE
(Version 2.1)**

Catalog No.
SAMPLE: 5 preps
GF-RD-025: 25 preps
GF-RD-100: 100 preps

High Yield and Purity
Fast and Easy purification
Reliable and Reproducible
Eluted RNA ready for use in downstream applications
No toxic or organic-based extraction required

Introduction

The **GF-1 Viral Nucleic Acid Extraction Kit** is designed for rapid and efficient purification of viral DNA/RNA from samples such as serum, plasma, body fluid or virus-infected cell culture supernatant. The purification is based on the usage of denaturing agents to provide efficient viral lysis, denaturation of proteins and subsequent release of DNA or RNA. Special buffers provided in the kit are optimized to enhance the binding of DNA or RNA onto a specially-treated glass filter membrane for efficient recovery of highly pure DNA or RNA.

Kit components

Product Catalog No.	5 Preps SAMPLE	25 Preps GF-RD-025	100 Preps GF-RD-100
Components			
GF-1 columns	5	25	100
Collection tubes	5	25	100
Buffer VL	1.5ml	6ml	24ml
Wash Buffer 1 (concentrate)*	1.5ml	7ml	28ml
Wash Buffer 2 (concentrate)*	1.7ml	9ml	36ml
Carrier RNA*	0.3mg	0.5mg	2 x 1mg
Elution Buffer	1.5ml	2 x 1.5ml	20ml
Proteinase K*	0.26ml	1.3ml	3 x 1.7ml
Handbook	1	1	1

* Please refer to **Reconstitution of Solutions** and **Storage and Stability**.

The **GF-1 Viral Nucleic Acid Extraction Kit** is available as 25 and 100 purifications per kit.

The reagents and materials provided with the kit are for research purposes only.

Additional Materials to be Supplied by User

Absolute Ethanol (>95%)

Reconstitution of Solutions

The bottle labeled **Wash Buffer 1** and **Wash Buffer 2** contain concentrated buffer which must be diluted with absolute ethanol (>95%) before use.

For **SAMPLE (5 preps)**,

Add **1.5ml** of absolute ethanol into the bottle labeled **Wash Buffer 1**.

Add **4ml** of absolute ethanol into the bottle labeled **Wash Buffer 2**.

Add **0.3ml** of **Elution Buffer** into the vial of **Carrier RNA** and mix well, prepare in 15 μ l aliquots to avoid repeated freeze-thaw cycles. Store at -20°C.

For **GF-RD-025 (25 preps)**,

Add **7ml** of absolute ethanol into the bottle labeled **Wash Buffer 1**.

Add **21ml** of absolute ethanol into the bottle labeled **Wash Buffer 2**.

Add **0.5ml** of **Elution Buffer** into the vial of **Carrier RNA** and mix well, prepare in 15 μ l aliquots to avoid repeated freeze-thaw cycles. Store at -20°C.

For **GF-RD-100 (100 preps)**,

Add **28ml** of absolute ethanol into the bottle labeled **Wash Buffer 1**.

Add **84ml** of absolute ethanol into the bottle labeled **Wash Buffer 2**.

Add **1ml** of **Elution Buffer** into one of the vials of **Carrier RNA** and mix well, prepare in 15 μ l aliquots to avoid repeated freeze-thaw cycles. Store at -20°C. Store the other vial of **Carrier RNA** at -20°C and dissolve in **Elution Buffer** only prior to use.

Store **Wash Buffer** at room temperature with bottle capped tight after use.

Storage and Stability

Store all solution at 20°C-30°C.

Store **Proteinase K** and **Carrier RNA** at -20°C.

Kit components are guaranteed to be stable for 12 months from the date of manufacture.

Buffer VL and **Wash Buffer 1** may exhibit salt precipitation due to cold temperature. If this occurs, simply warm the bottle at 55°C - 65°C with occasional mixing until completely dissolved.

Carrier RNA can only be thawed not more than once. Any remaining **Buffer VL** which contains **Carrier RNA** can only be stored at 4°C for not more than one week.

Chemical Hazard

Buffer VL and **Wash Buffer 1** contain guanidine salts which can be harmful when in contact with skin or swallowed. Always wear gloves and practice standard safety precautions. Do NOT disinfect guanidine or extraction waste in solutions containing bleach or any form of acid. To clean any items contaminated with the reagent, simply soak in detergent and water to remove all traces of guanidine before cleaning with bleach or acidic solutions.

Procedures

Reminder

·All steps are to be carried out at room temperature unless stated otherwise.

·**Wash Buffer 1** and **Wash Buffer 2** (concentrate) have to be diluted with absolute ethanol before use. Please refer to **Reconstitution of Solutions**.

·If precipitation forms in **Buffer VL**, incubate at 55°C - 65°C with occasional mixing until completely dissolved.

·Pre-set waterbath to 65°C.

·Prepare **Buffer VL** with **Carrier RNA** by adding 15 μ l of **Carrier RNA** into 200 μ l of **Buffer VL** per sample.

1. Sample lysis

Add 50 μ l of **Proteinase K** into 200 μ l of sample and mix thoroughly. Add 215 μ l of **Buffer VL** (containing **Carrier RNA**) and mix homogeneously by pulsed-vortexing. Incubate at 65°C for 10 min.

2. Addition of ethanol

Add 280 μ l of absolute ethanol. Mix immediately and thoroughly.

Mix immediately to prevent any uneven precipitation of nucleic acid due to high local ethanol concentrations.

3. Loading to column



Attention! Tips for Higher Yield

In order to obtain maximum yield, we strongly recommend users to fix the orientation of the column during centrifugation at all times. We recommend users to place the column which has a triangle mark on the edge, at a fixed position during centrifugation.

Transfer the sample into a column assembled in the collection tube (provided). Centrifuge at 5,000 x g for 1 min. Discard flow through.

4. Column washing 1

Wash the column with 500 μ l **Wash Buffer 1** and centrifuge at 5,000 x g for 1 min. Discard flow through.

Ensure that ethanol has been added into the Wash Buffer 1 before use (refer to Reconstitution of Solutions).

5. Column washing 2

Wash the column with 500µl **Wash Buffer 2** and centrifuge at 5,000 x g for 1 min. Discard flow through. Wash column again with 500µl **Wash Buffer 2** and centrifuge at maximum speed for 3 min.

Ensure that ethanol has been added into the Wash Buffer 2 before use (refer to Reconstitution of Solutions). Perform centrifugation for 3 min to remove ethanol completely.

6. DNA elution

Place the column into a clean microcentrifuge tube. Add 30-50µl of **Elution Buffer** or nuclease-free water directly onto column membrane and stand for 2 min. Centrifuge at 5,000 x g for 1 min to elute DNA/RNA.

Ensure that the Elution Buffer is dispensed directly onto the center of the membrane for complete elution. Store DNA at 4°C to -20°C or RNA at -20°C to -80°C.

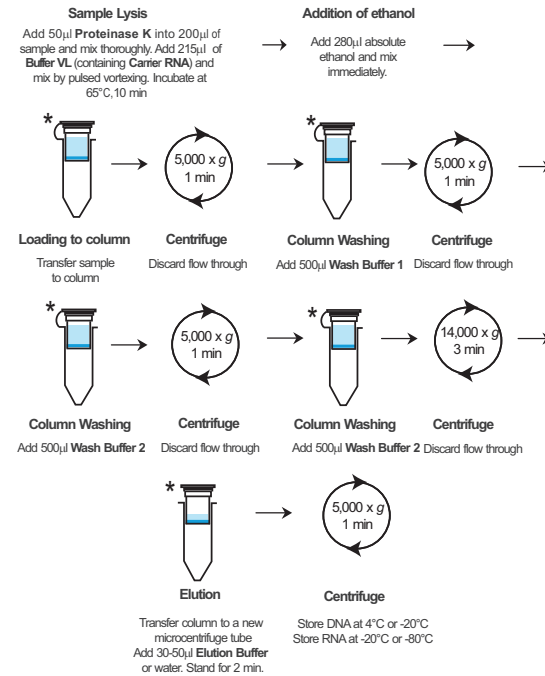
Troubleshooting

Please note that by not adhering to the recommended protocols, unsatisfactory results related to yield and quality of DNA/RNA may occur. If problems arise, please refer to the following:

Problem	Possibility	Suggestions
Low DNA/RNA yield	<i>Samples not fresh or not properly stored</i>	<i>Sample can only be thawed not more than once.</i>
	Carrier RNA is not added to Buffer VL	<i>Prepare Buffer VL with Carrier RNA as described in the procedures page.</i>
	<i>Low quality of Carrier RNA</i>	<i>Ensure that the Carrier RNA is aliquoted and can only be thawed not more than once. Please refer to page 3 for the "Storage and Stability".</i> <i>Ensure that any precipitate formed in Buffer VL is completely dissolved.</i>
	<i>Inefficient nuclease inhibition during sample lysis step</i>	<i>Ensure that Buffer VL is mixed homogeneously with the mixture of sample and Proteinase K.</i>
	<i>Ethanol is not added after sample lysis</i>	<i>Repeat purification with new sample.</i>
	<i>Column not placed at fixed orientation during centrifugation</i>	<i>Place the column which has a triangle mark on the edge, at a fixed position during centrifugation at all times.</i>
	Wash Buffer 1 and Wash Buffer 2 are reconstituted wrongly	<i>Please refer to "Reconstitution of Solutions". Repeat purification with new sample.</i>

Troubleshooting

Problem	Possibility	Suggestions
	Column is not dried before addition of Elution Buffer	Ensure that column is spun dried at maximum speed for 3 minutes after addition of Wash Buffer 2.
Poor performance of eluted DNA/RNA in downstream applications	RNA degraded	Process sample immediately or if sample is stored for later use, ensure that sample is thawed on ice. Use disposable plasticware and pipette tips. Ensure that the purification is performed in an RNase-free environment.
	Eluted DNA/RNA contains traces of ethanol	Ensure that the Column drying step is carried out prior to elution.
	Low concentration of eluted DNA/RNA	Reduce the amount of Elution Buffer but not less than 30µl
	The amount of added carrier RNA is inappropriate	User may optimize the amount of Carrier RNA to be added.



* We recommend users to place the column which has a triangle mark on the edge, at a fixed position during centrifugation.