vivantis

Nucleic Acid Extraction Kit HandBook

GF-1

TISSUE VIRAL NUCLEIC ACID EXTRACTION USER GUIDE (Version 1.0)

Catalog No.

SAMPLE: 5 preps

GF-TRD-050: 50 preps

GF-TRD-100 : 100 preps

High Yield and Purity

Fast and Easy purification

Reliable and Reproducible

Eluted nucleic acid ready for use in downstream applications

No toxic or organic-based extraction required

Introduction

GF-1 Tissue Viral Nucleic Acid Extraction Kit is designed for rapid and efficient purification of viral nucleic acid (DNA/RNA) from infected tissue. The purification is based on the usage of denaturing agents to provide efficient tissue lysis as well as 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. The extracted DNA or RNA can be used for various applications, including PCR, RFPL, and other molecular testings.

Kit components

Product Catalog No.	5 Preps SAMPLE	50 Preps GF-TRD-050	100 Preps GF-TRD-100
Components			
GF-1 columns	5	50	100
Collection tubes	5	50	100
Buffer VL1	1.5ml	12ml	24ml
Buffer VL2	1.5ml	12ml	24ml
Wash Buffer 1 (concentrate)*	1.5ml	14ml	28ml
Wash Buffer 2 (concentrate)*	1.7ml	17ml	36ml
Carrier RNA*	0.3mg	1mg	2 X 1mg
Elution Buffer	1.5ml	8ml	20ml
Proteinase K*	0.26ml	2 X 1.3ml	3 X1.7ml
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***** Please refer to **Reconstitution of Solutions** and **Storage and Stability.**

The **GF-1 Tissue Viral Nucleic Acid Extraction Kit** is available as 50 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 15µl aliquots to avoid repeated freeze-thaw cycles. Store at -20°C.

For **GF-TRD-050 (50 preps),**

Add **14ml** of absolute ethanol into the bottle labeled **Wash Buffer 1**.
Add **40ml** of absolute ethanol into the bottle labeled **Wash Buffer 2**.
Add **1ml** of **Elution Buffer** into the vial of **Carrier RNA** and mix well, prepare in 15µl aliquots to avoid repeated freeze-thaw cycles. Store at -20°C.

For **GF-TRD-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 the vial of **Carrier RNA** and mix well, prepare in 15µl 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.
Proteinase K and **Carrier RNA** are stable for up to 1 year after delivery when stored at room temperature or 4°C.
To prolong the lifetime of Proteinase K and carrier RNA, storage at -20°C is recommended. **Carrier RNA solution** (after being reconstituted) can only be thawed not more than once.
Kit components are guaranteed to be stable for 18 months from the date of manufacture. **Buffer VL2** 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.
Any remaining **Buffer VL2** which contains **Carrier RNA** can only be stored at 4°C for not more than one week.

Chemical Hazard

Buffer VL2 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 VL2,** incubate at 55°C - 65°C with occasional mixing until completely dissolved.
- Pre-set waterbath to 65°C.
- Prepare **Buffer VL2** with **Carrier RNA** by adding 15µl of **Carrier RNA** into 200µl of **Buffer VL2** per sample.

1. Sample lysis

a. Cut and weight 100mg of infected tissue sample into small pieces with a clean scalpel and load into a clean microcentrifuge tube. Add 200µl of **Buffer VL1** followed by 50µl of **Proteinase K** and mix thoroughly.

b. For extraction of viral DNA, add 200µl of **Buffer VL2.** For extraction of viral RNA, add 215µl of **Buffer VL2** (containing **Carrier RNA**). Mix homogeneously by pulsed-vortexing, incubate at 65°C for 30 min. Centrifuge at maximum speed for 1 min. Transfer supernatant into a new microcentrifuge tube.

2. Addition of ethanol

Add 280µl 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

Transfer the sample into a column (maximum 650µl) assembled in a clean collection tube (provided). Centrifuge at 5,000 x *g* for 1 min. Discard flow through. Repeat for the remaining sample from step 3.

4. Column washing 1

Wash the column with 500µl **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 the DNA/RNA may occur. If problems arise, please refer to the following:

Problem	Possibility	Suggestions
Low DNA/RNA yield	*Samples not fresh or not properly stored*	*Sample can only be thawed not more than once.*
	Carrier RNA *is not added to* **Buffer VL2**	*Prepare* **Buffer VL2** *with* **Carrier RNA** *as described in the procedures page.*
	Low quality of **Carrier RNA**	*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".*
		Ensure that any precipitate formed in **Buffer VL2** *is completely dissolved.*
	Inefficient nuclease inhibition during sample lysis step	*Ensure that* **Buffer VL2** *is mixed homogeneously with the mixture of sample and* **Proteinase K**.
	Ethanol is not added after sample lysis	*Repeat purification with new sample.*
	Wash Buffer 1 *and* **Wash Buffer 2** *are reconstituted wrongly*	*Please refer to 'Reconstitution of Solutions". Repeat purification with new sample.*

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*

Sample Preparation

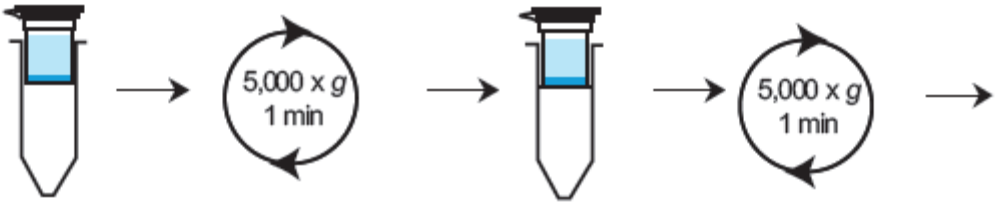
Cut and weight 100mg tissue and load into a clean microcentrifuge tube. Add 200µl **Buffer VL1.** Add 50µl **Proteinase K** and mix thoroughly.

Sample Lysis

For DNA, add 200µl of **Buffer VL2** For RNA, add 215µl of **Buffer VL2** (containing **Carrier RNA**) and mix by pulsed vortexing. Incubate at 65°C, 30 min. Centrifuge at max speed 1 min.

Additional of ethanol

Transfer supernatant into new tube. Add 280µl absolute ethanol and mix immediately.



Loading to Column

Transfer samples to column

Centrifuge

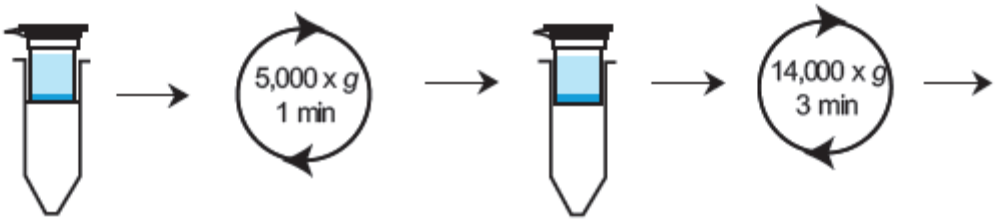
Discard flow through

Column Washing

Add 500µl **Wash Buffer 1.**

Centrifuge

Discard flow through



Column Washing

Add 500µl **Wash Buffer 2.**

Centrifuge

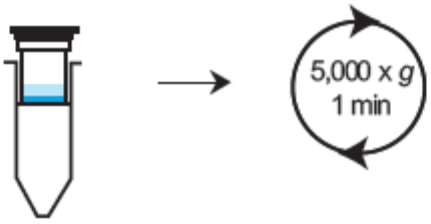
Discard flow through

Column Washing

Add 500µl **Wash Buffer 2.**

Centrifuge

Discard flow through



Elution

Transfer column to a new microcentrifuge tube. Add 30-50µl **Elution Buffer** or water. Stand for 2 min.

Centrifuge

Store DNA at 4°C or - 20°C
Store RNA at -20°C or -80°C

Contact : info@vivantechnologies.com