vivantis

Nucleic Acid Extraction Kit HandBook

96 GF-1

96-WELL TOTAL RNA EXTRACTION KIT USER GUIDE (Version 1.1)

Catalog No.
GF-96-R05: 96 x 5plates
GF-96-R10: 96 x 10plates

Up to 90% recovery of RNA

Purification process takes less than 60 minutes

No organic-based extraction required

Highly pure total RNA ready to use for routine molecular biology applications

Introduction

The **GF-1 96-well Total RNA Extraction Kit** is designed for fast extraction of total RNA from biological samples, including Gram negative and positive bacteria. The kit uses a specially-treated glass filter membrane fixed into a 96-well format plate to efficiently bind RNA in the presence of high salt. The total RNA in the lysate of bacteria is selectively bound on each column of the 96-well plate and other impurities such as proteins and salts do not bind. No ethanol precipitation is required. Purified total RNA can be used for many downstream applications such as RT-PCR, Northern Blot, cDNA synthesis. The kit can be applied on animal, fungi and some of the plant samples. However, the kit may not suitable for RNA extraction from the plants containing high levels of secondary metabolites, polyphenols and polysaccharides.

Kit component

Product	5 x 96	10 x 96
Catalog No.	GF-96-R05	GF-96-R10
Components		
GF-1 96-well DNA Binding Plate	5	10
Deep Well Collection Plate	10	20
96-well Storage Plate	5	10
Sealing Film	15	30
Lysis Buffer BG	200ml	2 x 200ml
Wash Buffer 1 (concentrate)*	2 x 90ml	4 x 90ml
Wash Buffer 2 (concentrate)*	60ml	2 x 60ml
RNase-free Water	25ml	2 x 25ml
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* Please refer to **Reconstitution of Solutions** and **Storage and Stability** before using this kit.

The **GF-1 96-well Total RNA Extraction Kit** is available as 5 x 96 and 10 x 96 purifications per kit. The reagents and materials provided with the kit are for research purposes only

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Additional Materials to be Supplied by User

Absolute Ethanol (>95%)
Lysozyme (Bacterial RNA Purification)

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 **GF-96-R05 (5 x 96),**

Add **60ml** of absolute ethanol into the bottle labeled **Wash Buffer 1** only prior to use**.**
Add **120ml** of absolute ethanol into the bottle labeled **Wash Buffer 2** only prior to use**.**

For **GF-96-R10 (10 x 96),**

Add **60ml** of absolute ethanol into the bottle labeled **Wash Buffer 1** only prior to use**.**
Add **120ml** of absolute ethanol into the bottle labeled **Wash Buffer 2** only prior to use**.**

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

Storage and Stability

Store all solutions at 20°C – 30°C.
Kit componenets are guaranteed to be stable for 18 months from the date of manufacture.
Lysis Buffer BG and **Wash Buffer 1** may exhibit salt precipitation due to cold temperature.
If this occurs, simply warm the bottles at 55°C - 65°C with occasional mixing until the precipitate is completely dissolved.

Chemical Hazard

Lysis Buffer BG 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 other 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** (concentrate) has to be diluted with absolute ethanol before use. Please refer to **Reconstitution of Solutions**.
- If precipitation forms in **Lysis Buffer BG**, incubate at 55 - 65°C with occasional mixing until precipitate is completely dissolved.
- Users are recommended to use a multichannel pipette.
- For centrifugation based method, there is a minimum height requirement of 75mm for apparatus to hold the assembly of 96-Well DNA Binding Plate and Deep Well Collection Plate.

Pre-set incubator / oven to 65°C.
Pre-set another incubator to 55°C.

1. Tissue preparation

Transfer 1-3ml bacteria culture grown overnight or culture grown to log phase into **Deep Well Collection Plate** by centrifugation at 5000 x *g* for 1 min. Discard supernatant.

2. Lysozyme treatment

For Gram-negative bacterium, add 100µl lysozyme (400µg/ml) into the pellet.
For Gram-positive bacterium, add 100µl lysozyme (3mg/ml) into the pellet.
Suspend the pellet and lysozyme solution thoroughly and incubate at 37°C for 5 mins.

3. Homogenization

Add 300µl of **Lysis Buffer BG** and seal the **Deep Well Collection Plate**, close with **Sealing Film**. Mix all solutions by inverting immediately.

4. Addition of Ethanol

Add 0.5 volume of ethanol into **Deep Well Collection Plate**, close with **Sealing Film** and mix thoroughly.

5. Please refer to Part A for Centrifugation Protocol
Please refer to Part B for Vacuum Protocol

Part A: Centrifugation Protocol

6. Loading to binding plate

Remove **Sealing Film**. Transfer the samples carefully into the **96-well Binding Plate** assembled into a **Deep Well Collection Plate.** Do not wet the rims of the wells to avoid aerosol formation during centrifugation. Centrifuge at 5000 x *g* for 2 mins. Discard flow through.

7. Plate washing 1

Add 500µl of **Wash Buffer 1** into each well carefully. Centrifuge at 5000 x *g* for 1 min. Discard flow through.

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

8. Plate washing 2

Add 500µl of **Wash Buffer 2** into each well carefully. Centrifuge at 5000 x *g* for 1 min. Discard flow through.
Ensure that ethanol has been added into Wash Buffer 2 before use (refer to Reconstitution of Solutions).

9. Plate drying

Centrifuge the **96-well DNA Binding Plate** at 5000 x *g* for 2 mins or dry the **96-well DNA Binding Plate.**
It is essential to remove traces of ethanol as it will inhibit downstream applications.

10. RNA elution

Place the **96-well DNA Binding Plate** onto a clean **96-well Storage Plate**. Add 50µl of **RNase-free Water** to each well and stand for 2 mins. Centrifuge at 5000 x *g* for 30 secs. Total RNA is ready to use or store at -80°C.

Part B: Vacuum Protocol

6. Loading to binding plate

Place the **96-well DNA Binding Plate** on top of the vacuum manifold. Place a waste tray or a **Deep well Collection Plate** underneath to collect the waste. Remove the **Sealing Film.** Transfer the lysates carefully into the **96-well DNA Binding Plate.** Do not wet the rims of the wells to avoid aerosol formation. Apply vacuum at 10-20 inches Hg for 3-5mins until all samples have passed.
Ensure that the 96-well DNA Binding Plate is fitted properly on the vacuum manifold. It is necessary to discard the flow through at all times after collection of each buffer flow through, and to blot the top of the plate on paper towels.

7. Plate washing 1

Add 500µl of **Wash Buffer 1** into each well carefully. Apply vacuum at 10 inches Hg until the buffer has passed through the **96-well DNA Binding Plate.**
Ensure that ethanol has been added into Wash Buffer 1 before use (refer to Reconstitution of Solutions).

8. Plate washing 2

Add 500µl of **Wash Buffer 2** into each well and apply vacuum at 10 inches Hg until the buffer has passed through.
Ensure that ethanol has been added into Wash Buffer 2 before use (refer to Reconstitution of Solutions).

9. Plate drying

Apply vacuum at 10 inches Hg for additional 10mins, or dry the **96-well DNA Binding Plate** at 65°C in an incubator / oven for 10mins.
It is essential to remove traces of ethanol as it will inhibit downstream applications.

10. DNA elution

Place the **96-well Storage Plate** on top of the waste tray or **Deep Well Collection Plate**, which are both placed inside the vacuum manifold. Place the **96-well DNA Binding Plate** on the vacuum manifold. Add 50µl of

RNase-free Water to each well and stand for 1 min. Apply vacuum at 10 inches Hg for 2mins. Total RNA is ready to use or store at -80°C.

Ensure that the RNase-free Water is dispensed directly onto the center of membrane for complete elution. Store RNA at -80°C as RNA may degrade in the absence of a buffering agent.

Troubleshooting

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

Problem	Possibility	Suggestions
Low RNA yield	*Insufficient sample disruption or homogenization*	*Tissue or plant samples need to be till become fine powder.* *Homogenize tissue or plant sample with rotor-stator homogenizer or pass the homogenate through a 18-21 gauge needle several times till visible tissue or plant fragment is eliminated.* .
	Sample not fresh or not properly stored	*For long term storage of tissues, keep at -70°C.*
	Addition of ethanol was neglected	*Repeat purification again with new samples.*
	Well in binding plate is clogged	*Do not use more than recommended amounts of sample material. If any undigested material remains, spin at maximum speed for 5mins to remove tissue lysate and transfer supernatant in a new* **Deep Well Collection Plate.** *Ensure that* **Wash Buffer** *is applied to the binding plate.*
	Proteinase K *activity is decreased*	*Avoid repeated freeze thaw cycles of* **Proteinase K** *solutions. Ensure that* **Proteinase K** *is stored at -20°C.*
	Wash Solution *reconstituted wrongly.*	*Please refer to "Reconstitution of Solutions". Repeat purification with new samples.*

	DNA Binding Plate *is not dried before addition of* **Elution Buffer**	*Ensure that the* **DNA Binding Plate** *is spin dried by centrifugation at 5700 x g for 10mins or dried at 65°C for 10mins, or apply vacuum for additional 10mins to remove traces of ethanol completely.*
	Elution is not performed properly	*Pre-heat* **Elution Buffer** *at 65-70°C before eluting DNA.*

Problem	**Possibility**	**Suggestions**
Low purity ($A_{260/280}$)	**Proteinase K** *activity is decreased*	*Please refer to problem "Low DNA yield".*
	Incomplete protein denaturation	*Use fresh* **Proteinase K** *and extend incubation time until lysate clears.*
DNA degradation smearing	*DNA sheared during purification*	*Add the addition of* **Buffer ACL** *and* **Proteinase K**, *avoid vigorous mixing and pipetting. Use cut-off tip if lysate appeared viscous.*
	Sample too old	*DNA already degraded in old sample.*
	Sample frozen and thawed repeated	*Avoid repeated freeze-thaw cycles.*
Poor performance of eluted DNA in downstream applications	*Eluted DNA contains traces of ethanol*	*Ensure that the plate drying step is carried out prior to elution.*
	TE buffer is used to elute DNA. EDTA in TE buffer may inhibit subsequent enzymatic reaction.	*Use* **Elution Buffer** *or water with pH range of 7.0 – 8.5.*