GF-1

SOIL SAMPLE DNA EXTRACTION KIT USER GUIDE

(Version 5.3)

Catalog No.
GF-SD-005: 5 prep
GF-SD-025: 25 prep

High Yield and Purity

Fast and Easy purification

Reliable and Reproducible

Eluted DNA ready for use in downstream applications

No toxic or organic-based extraction required

Introduction

The **GF-1 Soil Sample DNA Extraction Kit** is designed for the rapid and efficient purification of bacteria DNA from soil samples without the need for precipitation or organic extraction. The kit uses a high pure specially-treated silica-based material fixed into a column to efficiently bind DNA in the presence of high salt. The kit applies the principle of a mini-column spin technology and the use of optimized buffers to ensure that only DNA is isolated while cellular protein, humic acid, metabolites, salts and other low molecular weight impurities are removed during the subsequent washing steps. High quality DNA eluted in low salt buffer or water is ready to use in downstream applications such as restriction enzyme digestion, PCR and other manipulations.

Kit components

Product Catalog No.	5 Preps SAMPLE	25 Preps GF-SD-025
Components		
GF-1 DNA Binding Columns	5	25
Collection tubes	5	25
Glass Beads	2.5g	12.5g
Buffer SL1	5ml	25ml
Buffer SL2	0.5ml	2ml
Buffer SB	3.5ml	17ml
SPW Wash Buffer 1	2ml	10ml
SPW Wash Buffer 2	2.4ml	17ml
Elution Buffer	1ml	5ml
Handbook	1	1

* Please refer to **Reconstitution of Solutions** and **Storage and Stability** before using this kit.

The **GF-1 Soil Sample DNA Extraction Kit** is available as 25 purifications per kit.

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

Additional Materials to be provided by Users

Absolute Ethanol (>95%)
RNase A (25mg/ml)

Reconstitution of Solutions

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

For **SAMPLE (5 preps)**,

Add **2ml** absolute ethanol into the bottle labeled **SPW Wash Buffer 1**.
Add **5.6ml** absolute ethanol into the bottle labeled **SPW Wash Buffer 2**.

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

Add **10ml** absolute ethanol into the bottle labeled **SPW Wash Buffer 1**.
Add **40ml** absolute ethanol into the bottle labeled **SPW Wash Buffer 2**.

Storage and Stability

Store all solutions at 20-30°C unless stated otherwise.
Some buffers (**Buffer SL1** and **Buffer SB**) may exhibit salt precipitation due to cold temperature. If this occurs, simply warm the bottles at 55°C - 65°C with occasional mixing until precipitate is completely dissolved.

Chemical Hazard

Buffer SB contains 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 solution

Procedures

Reminder

- All steps should be carried out at room temperature unless stated otherwise.
- **SPW Wash Buffer 1** and **SPW Wash Buffer 2** (concentrate) has to be diluted with absolute ethanol before use. Please refer to **Reconstitution of Solutions**.

Pre-set waterbath at 70°C.

Pre-set another waterbath at 37°C (Optional for removal of RNA).

1. Aliquot 0.5g **Glass Beads** into 2ml microcentrifuge tube. Add 250mg (200mg–500mg) soil sample into the 2ml microcentrifuge tube containing **Glass Beads**.

2. Add 1ml **Buffer SL1** into tube and invert to mix well. Vortex tube at maximum speed for 5 min. Incubate tube at 70°C for 10 min. Mix the sample twice by vortexing during incubation.

3. Centrifuge at 10,000 x *g* for 5 min to pellet the soil particle. Transfer 650µl of the supernatant into a new microcentrifuge tube.
Be careful not to transfer any debris into the new tube.

Optional: Removal of RNA
If RNA-free DNA is required, add 4µl of DNase-free RNase A (25mg/ml) into the sample and mix thoroughly by tap-vortexing. Incubate at 37 °C for 10 min.

4. Add 65µl **Buffer SL2** into the sample and mix thoroughly by inverting the tube. Incubate the sample on ice for 5 min.

5. Centrifuge at maximum speed (14.000 x *g*) for 5 min to pellet the soil particle. Transfer 600µl of the supernatant into a new 1.5ml or 2ml microcentrifuge tube.
Be careful not to transfer any debris into the new tube.If the supernatant is unclear, centrifuge again at maximum speed for another 5 min.

6. Add 600µl of **Buffer SB** into the sample and mix thoroughly by pipette mixing.

7. Transfer 600µl of the sample into a column assembled in a clean collection tube (provided). Centrifuge at 10,000 x *g* for 1 min. Discard flow through.
Repeat for any remaining sample from step 7.

8. Wash the column with 650µl **SPW Wash Buffer 1** and centrifuge at 10,000 x *g* for 1 min. Discard flow thorough.
Ensure that ethanol has been added into DNA Wash Buffer before use (refer to Reconstitution of Solutions).

9. Wash the column with 650µl **SPW Wash Buffer 2**. Centrifuge the column at 10,000 x *g* for 1 min. Discard flow through. Repeat washing with 650µl of **SPW Wash Buffer 2** again. Discard flow thorough.
Ensure that ethanol has been added into DNA Wash Buffer before use (refer to Reconstitution of Solutions).

10. Centrifuge the column at maximum speed (14,000 x *g*) for 2 min to remove all traces of ethanol.
This step is critical in removing traces of ethanol that will interfere with downstream applications.

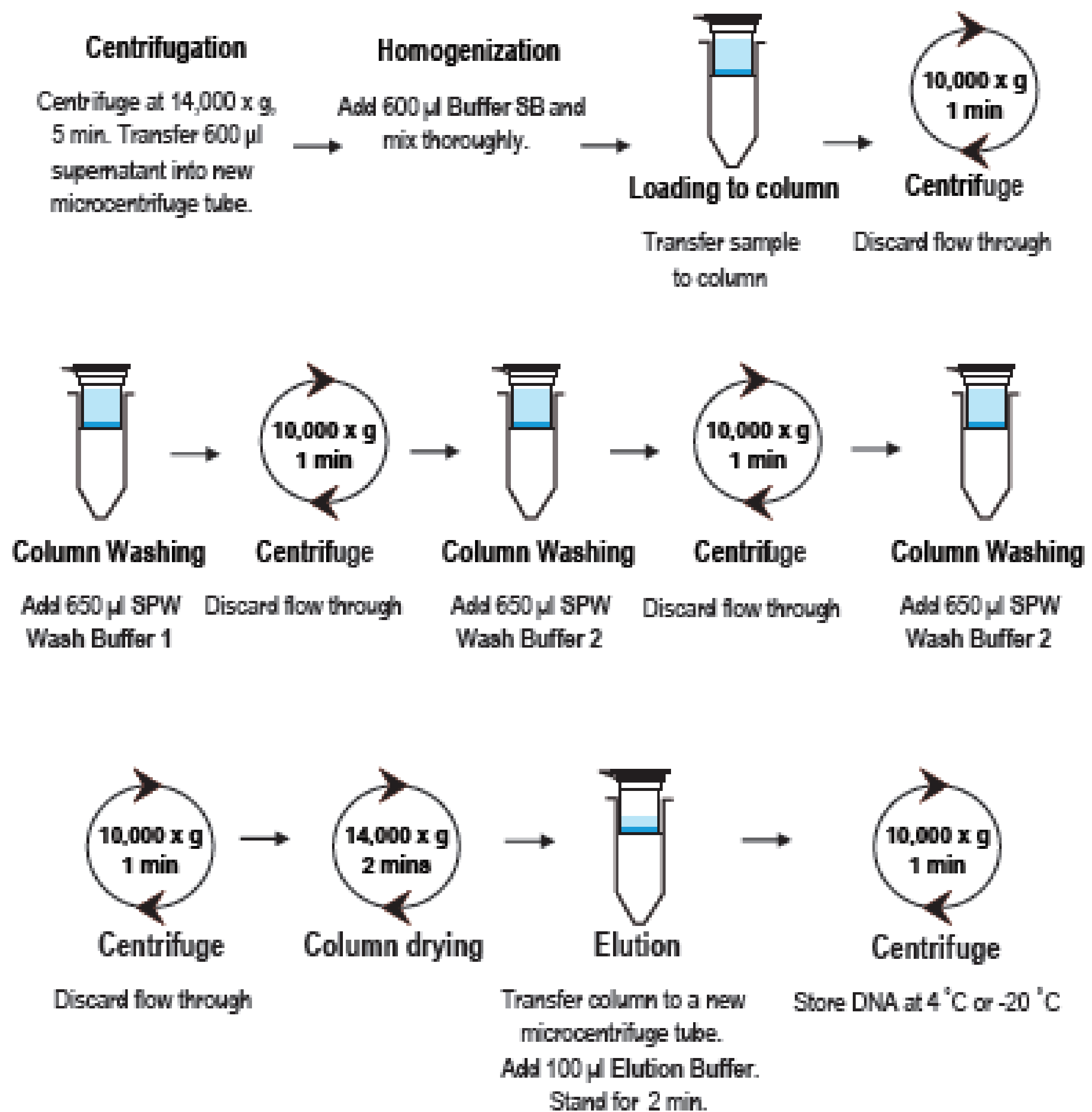
11. Place the column into a clean 1.5ml microcentrifuge tube. Add 100µl of preheated **Elution Buffer** directly onto the center of the membrane and stand for 2 min. Centrifuge at 10,000 x *g* for 1 min to elute DNA.
Ensure that the Elution Buffer is dispensed directly onto the center of membrane for complete elution. TE buffer can also elute DNA although EDTA may inhibit enzymatic reactions. If water is used for eluting DNA, maximum elution efficiency is achieved between pH7.0 and 8.5. Store DNA at -20°C as DNA 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 DNA yield	*Low amount of DNA in soil sample*	*Use a few more* **Glass Beads** *for one sample.*
	Poor homogenization of sample	*Ensure that the sample is mix with* **Buffer SL1** *and* **Glass Beads** *thoroughly*
		Prolong bead vortexing time to ensure the samples are fully homogenized.
	SPW Wash Buffer *is reconstituted Wrongly*	*Please refer to "Reconstitution of Solutions". Repeat purification with a new sample.*
	Column clogged	*Check the centrifugal force and increase the time of centrifugation.*
		Ensure that not to transfer any debris into the column.
	Column is not dried before addition of **Elution Buffer**	*Ensure that column is spun dried at maximum speed for 2 min after column washing steps.*
	Elution is not performed properly	*Incubate column at 70°C for 5 min before adding into column.*
		Ensure that the **Elution Buffer** *used is a low salt buffer or water with a pH range of 7.0 – 8.5.*

Problem	Possibility	Suggestions
Low purity $_{(A260/280)}$ or $_{(A260/230)}$ ratio	*Poor homogenization of sample*	*Please refer to problem "Low DNA yield"*
	Presence of humic acids	*Wash the column with extra* **Buffer SB**, *incubate for 2 min and centrifuge for 2 min.*
	Columns have traces of ethanol	*Dry the column at maximum speed for 2 min after washing steps.*
Poor performance of eluted DNA in downstream applications	*Eluted DNA contains traces of ethanol*	*Centrifuge the column at maximum speed for 2 min after washing steps.*
	Inefficient elimination of inhibitors	*Wash the column with extra* **Buffer SB**, *incubate for 2 min and centrifuge for 2 min.*
	TE buffer is used to elute DNA. EDTA in TE buffer may inhibit subsequent enzymatic reaction	*Use* **Elution Buffer** *or water with a pH range of 7.0 - 8.5.*
	Excess amount of DNA inhibits PCR	*Check the DNA amount through spectrophotometer or gel electrophoresis. Dilute the DNA if necessary.*
	DNA degraded	*Store extracted DNA at -20°C as DNA may degrade in the absence of a buffering agent.*



Contact : info@vivantechnologies.com