Nucleic Acid Extraction Kit HandBook

GF-1

SOIL SAMPLE DNA EXTRACTION KIT USER GUIDE

(Version 5.1)

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 up to 1 gram of soil samples without the need for precipitation or organic extraction. The kit uses a 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	10	50
Glass Beads	3g	15g
HTR Reagent	1.2ml	6ml
Buffer DS	0.6ml	3ml
Buffer SLX Mlus	6ml	30ml
P2 Buffer	2 x 1.5ml	12.5ml
XP1 Buffer	4ml	20ml
SPW Wash Buffer*	2ml	10ml
Elution Buffer	2 x 1.5ml	15ml
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* 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%)
Isopropanol
RNase A (25mg/ml)

Reconstitution of Solutions

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

For **SAMPLE (5 preps)**,

Add **8ml** absolute ethanol into the bottle labeled **SPW Wash Buffer**

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

Add **40ml** absolute ethanol into the bottle labeled **SPW Wash Buffer**

Storage and Stability

Store all solutions at 20-30°C unless stated otherwise.
Some buffers 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

XP1 Buffer 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** (concentrate) has to be diluted with absolute ethanol before use. Please refer to **Reconstitution of Solutions**.
- Mix the bottle of **HTR Reagent** thoroughly by vortexing for 30 sec before use to make sure particles are thoroughly resuspended.

Pre-set waterbath at 70°C.

Pre-set another waterbath at 95°C (Optional for Gram Positive bacteria)

1. Add 0.2-1g of soil sample into a 2ml microcentrifuge tube containing 500mg of **Glass Beads**. Add 1ml **Buffer SLX Mlus** into tube and vortex at maximum speed for 3-5 min to homogenize the sample thoroughly. Add 100µl of **Buffer DS** and vortex thoroughly. Incubate at 70°C for 10 min. Mix the sample twice by vortexing during incubation. For DNA isolation from Gram positive bacteria, incubate the sample again at 95 °C for 2min.

2. Centrifuge at 5,000 x *g* for 3 min at room temperature. Transfer 800µl of the supernatant into a new microcentrifuge tube. Add 270µl of **Buffer P2** into the sample tube and mix thoroughly by vortexing. Incubate the sample on ice for 5 min.

3. Centrifuge at maximum speed (14,000 x *g*) for 3 min to pellet the soil particle. Transfer all nof the supernatant into a new microcentrifuge tube.
Be careful not to transfer any debris into the new tube.

4. Add 0.7volume of isopropanol and mix by inverting the tubes 20-30 times. If the soil sample contain low amount of DNA, incubate the sample at -20 °C for 1 hour.

5. Centrifuge at maximum speed for 10 min at 4 °C. Discard the supernatant carefully without dislodge the DNA pellet. Invert the tube on the paper towel to drain the remaining liquid for 1 min. It is not necessary to dry the DNA pellet

6. Add 200µl of **Elution Buffer** and mix by pulsed-vortexing. Incubate at 70°C for 10-20 min to dissolve DNA pellet.

7. Add 100µl of **HTR Reagent** and mix thoroughly by vortexing for 10 sec. Incubate at room temperature for 2 min.
Mix the bottle of HTR Reagent vigorously before use.

8. Centrifuge at maximum speed for 2 min.

9. Transfer the supernatant into a clean 1.5ml microcentrifuge tube. If the supernatant still shows dark colour from the soil sample at this point, perform the HTR extraction again by repeating step 7-9.

Optional: Removal of RNA
If RNA-free DNA is required, add 2µl of RNase A (25mg/ml) into the sample and mix thoroughly by vortexing. Incubate at 37 °C for 10 min.

10. Add equal volume of **XP1 Buffer** to the cleared sample and mix thoroughly by vortexing. If the sample volume from step 9 is 300µl, then add 300µl **XP1 Buffer.**

11. Insert a column into a collection tube. Transfer 600µl of sample into the column. Centrifuge at 10,000 x g for 1 min. Discard flow through. Repeat for the remaining sample from step 10.

12. Add 300µl of **XP1 Buffer** and centrifuge at 10,000 x *g* for 1 min. Discard flow thorough and collection tube.

13. Insert the column into a new collection tube. Add 700µl **SPW Wash Buffer** and centrifuge at 10,000 x *g* for 1 min. Discard flow thorough. Repeat washing with 700µl of **SPW Wash Buffer** again. Discard flow thorough.
Ensure that ethanol has been added into DNA Wash Buffer before use (refer to Reconstitution of Solutions).

14. Centrifuge the column at maximum speed for 2 min to remove all traces of ethanol.
This step is critical in removing traces of ethanol that will interfere with downstream applications.

15. Place the column into a clean microcentrifuge tube. Add 30-100µl of **Elution Buffer**, directly onto the center of the membrane. Incubate at 70°C for 5 min. Centrifuge at maximum speed for 1 min to elute DNA. Repeat elution step with a second 30-100µl of Elution Buffer.
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	*Soil sample is not stored properly*	*Soil sample should be stored at 4°C or -20°C.*
	Poor homogenization of sample	*Ensure that the sample is mix with* **Buffer SLX** *and* ***Glass Beads*** *thoroughly* Prolong bead beating time to ensure the sample 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* **XP1 Buffer** *is applied to 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 65°C for 5 min after addition of* **Elution Buffer**. *Ensure that the* **Elution Buffer** *used is a low salt buffer or water with a pH range of 7.0 – 8.5.*
	Column matrix lost binding capacity during storage	*Add 100µl 3M NaOH to the column prior to loading the sample .Centrifuge at 10,000 x g for 30 seconds. Discard the filtrate.*

Problem	Possibility	Suggestions
Low purity (A260/280)	*Poor homogenization of sample*	*Please refer to problem "Low DNA yield"*
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	*Ensure that the sample is mixed with* **HTR Reagent** *thoroughly. Repeat purification with a new sample.*
	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.*