vivantis

Nucleic Acid Extraction Kit HandBook

GF-1

FUNGUS DNA EXTRACTION USER GUIDE (Version1.0)

Catalog No.

SAMPLE: 5 preps

GF-FU-050: 50 preps

GF-FU-100:100 preps

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 Fungus DNA Extraction Kit** is designed for rapid and efficient purification of genomic DNA from a wide variety of fungus samples without the need for precipitation or organic extractions. The kit applies the principle of a mini-column spin technology and the use of optimized buffers to ensure only DNA is isolated and bind to membrane of columns in the presence of high salt while cellular proteins, metabolites and other low molecular weight impurities are removed during the subsequent washing steps. High purity genomic DNA is eluted in water or low salt buffers has a $A_{260/280}$ ratio between 1.7 and 2.0, making it ready to use in many routine molecular biology applications such as restriction enzyme digestion, Southern blotting, DNA fingerprinting, PCR and other manipulations.

Kit components

Product	5 Preps	50 Preps	100 Preps
Catalog No.	SAMPLE	GF-FU-050	GF-FU-100
Components			
GF-1 columns	5	50	100
Collection tubes	5	50	100
Fungus Lysis Buffer **(Buffer FuL)**	2 x 1ml	18ml	36ml
Fungus Genomic Binding Buffer **(Buffer FuB)**	4ml	35ml	70ml
Wash Buffer (concentrate)*	2.4ml	24ml	2 x 24ml
Elution Buffer	1.5ml	10ml	20ml
Proteinase K*	0.11ml	1.05ml	2 x 1.05ml
Handbook	1	1	1

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

The **GF-1 Fungus DNA 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%)
RNase A (DNase-free) (20mg/ml)

Reconstitution of Solutions
The bottle labeled **Wash Buffer** contains concentrated buffer which must be diluted with absolute ethanol (>95%) before use.

For **SAMPLE (5 preps),**
Add **5.6ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

For **GF-FU-050 (50 preps),**
Add **56ml** of absolute ethanol into the bottle labeled **Wash Buffer**.

For **GF-FU-100 (100 preps)**,
Add **56ml** of absolute ethanol into one of the bottles labeled **Wash Buffer**.
Add **56ml** of absolute ethanol into the other bottle labeled **Wash Buffer** only prior to use.

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

Storage and Stability
Store solutions at 20°C - 30°C.
Proteinase K is stable for up to 1 year after delivery when stored at room temperature or 4°C. To prolong the lifetime of Proteinase K, storage at -20°C is recommended.
Kit components are guaranteed to be stable for 18 months from the date of manufacture. **Buffer FuB** 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.

Chemical Hazard
Buffer FuB 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 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 **Buffer FuB**, incubate at 55°C - 65°C with occasional mixing until completely dissolved.

Pre-set waterbath to 65°C.

Pre-heat **Elution Buffer** at 65°C (optional).

A. Fungus Sample in plate or at site

1. **Homogenization**
   a. Collect 30mg of fungus sample into a new microcentrifuge tube. Add 280µl of **Buffer FuL** to the fungus sample. Mix thoroughly by vortexing the tube for 30 sec to obtain a homogenous solution.
   b. If the fungus sample is viscous or the fungus sample cannot be stick to tube when doing fungus sample collection, first add 280µl of **Buffer FuL** to a new microcentrifuge tube, collect the fungus sample using inoculation loop and mix the fungus sample by stirring the inoculation loop in the **Buffer FuL** in the tube. Collect the fungus sample until the lysate is cloudy. Proceed to step 2.

B. Fungus Culture

1. **Homogenization**
   a. Pellet 1-3ml of fungus culture grown overnight or culture grown to log phase by centrifugation at 6,000 x *g* for 5 min at room temperature. Decant the supernatant completely.
   b. Add 280µl **Buffer FuL** to the pellet and resuspend the cells completely by pipetting up and down or vortexing the tube gently for 30 sec to obtain a homogenous solution. Proceed to step 2.
   Thorough removal of supernatant is essential as residual culture media may affect both yield and purity. Ensure complete cell suspension. Lysis will not occur if clumps of fungus remain following an inefficient resuspension procedure.

2. **Sample lysis**

Add 20µl of **Proteinase K** and mix thoroughly by inverting tube. Incubate at 65°C for 30 min in a shaking waterbath or with occasional mixing every 5 min during incubation to ensure thorough digestion of the sample.

3. **Centrifugation**

Centrifuge at maximum speed (≥14,000 x *g*) for 5 min to precipitate any insoluble/undigested materials. Transfer the supernatant containing the DNA into a clean microcentrifuge tube.

Extend centrifugation time if solids are not completely spun down.

Optional: Removal of RNA

If RNA-free DNA is required, add 20µl of RNase A (DNase-free, 20mg/ml). Mix and incubate at 37°C for 5 min.

Residual RNA fragments will be removed during column washing.

4. **Homogenization**

Add 2 volumes (~600µl without RNase A treatment, ~640µl with RNase A treatment) of **Buffer FuB** and mix thoroughly until a homogeneous solution is obtained by inverting tube several times. Incubate at 65°C for 10 min.

Precipitation may occur due to high DNA content. Lysate should be clear upon mixing and incubation at 65°C.

5. **Addition of ethanol**

Add 200µl of absolute ethanol. Mix immediately and thoroughly.

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

6. **Loading to column**

Transfer the sample into a column (max. 650µl) assembled in a clean collection tube (provided). Centrifuge at 10,000 x *g* for 1 min. Discard flow through.

Repeat for the remaining sample from step 6.

If column clogs, add 200µl Buffer FuB into column and centrifuge as above.

7. **Column washing**

Wash the column with 650µl **Wash Buffer** and centrifuge at 10,000 x *g* for 1 min. Discard flow through. Repeat washing with 650µl **Wash Buffer**.

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

8. **Column drying**

Centrifuge the column at 10,000 x *g* for 1 min to remove residual ethanol.

This step has to be carried out to remove all traces of ethanol completely as residual ethanol can affect the quality of DNA and may subsequently inhibit enzymatic reactions.

9. **DNA elution**

Place the column into a clean microcentrifuge tube. Add 50 of preheated **Elution Buffer**, TE buffer or sterile water directly onto column membrane and stand for 2 min. Centrifuge at 10,000x *g* for 1 min to elute DNA. Store DNA at 4°C or -20°C.

Ensure that the Elution Buffer is dispensed directly onto the center of the membrane for complete elution. TE Buffer can also elute DNA although EDTA may inhibit subsequent 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:

Problems	Possibility	Suggestions
Low DNA yield	*Sample not thoroughly homogenized*	*Ensure that tissues are completely homogenized in* **Buffer FuL**
	Sample not lysed completely	*Ensure that tissues are completely homogenized in* ***Buffer FuL*** *and mix sample frequently during incubation in absence of a waterbath shaker.*
	Low elution efficiency	*Pre-heat* **Elution Buffer** *to 65°C-70°C before eluting DNA.*
	Column clogged	*Refer to Problems under 'Column clogged'.*
Low purity	*Incomplete protein denaturation*	*Extend incubation time until lysate is clear.*
	RNA contamination	*Add RNase A to the sample as indicated in the protocol. Ensure that RNase A used has not been repeatedly frozen and thawed. If necessary prepare a fresh stock*
No DNA eluted	*Inappropriate elution buffer*	*Ensure that the* ***Elution Buffer*** *used is a low salt buffer or water with a pH range of 7.0-8.5*

Troubleshooting

Problems	Possibility	Suggestions
Column clogged	*Overloading column/ starting material too high*	*Do not use more than 30mg of sample material. If any undigested material remains, centrifuge tissue lysate and transfer supernatant into a new microcentrifuge tube.*
	Sample not thoroughly homogenized	*Vortex sample in **Buffer FuL** prior to addition of **Proteinase K***
DNA degradation/ smearing	*DNA sheared during purification*	*After the addition of* **Buffer FuL** *and* **Proteinase K**, *avoid vigorous mixing and pipetting. Mix gently by inverting tube.*
	Nuclease contamination	*Use sterilized glassware, plasticware and wear gloves.*
		*Ensure that the tissue is completely homogenized in **Buffer FuL** and **Proteinase K***
Poor performance of eluted DNA in downstream applications	*Eluted DNA contains traces of ethanol*	*Ensure that the Column drying step is carried out prior to elution.*

Homogenization

A- Collect 30mg of fungus sample into a new microcentrifuge tube
B- Pellet 1-3ml of fungus culture at 6000 x *g* for 5 min.

Tissue Lysis

Add 280µl **Buffer FuL**.
Vortex sample 30 sec.
Add 20µl **Proteinase K**.
Incubate 65°C, 30 min.

Centrifugation

Centrifuge at 14,000 - 16,000 x g, 5 min.
Transfer supernatant into new microcentrifuge tube.

Optional: Removal of RNA

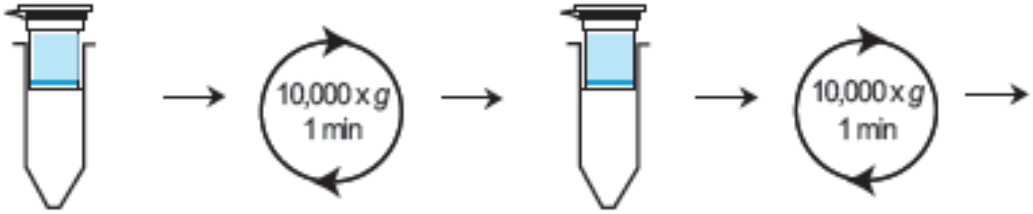
Add 20µl RNase A
Incubate 37°C, 5 min.

Homogenization

Add 2 volumes **Buffer FuB** and mix thoroughly.
Incubate 65°C, 10 min.

Additional of ethanol

Add 200µl absolute ethanol and mix immediately.



Loading to column

Transfer sample to column

Centrifuge

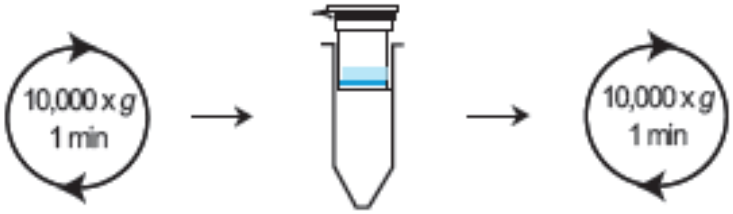
Discard flow through

Column Washing

Add 650µl **Wash Buffer**

Centrifuge

Discard flow through



Column Drying

Elution

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

Centrifuge

Store DNA at 4°C or -20°C

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