



**vivantis**  
Nucleic Acid Extraction Kit HandBook

***GF-1***

**FOOD DNA EXTRACTION KIT  
USER GUIDE (Version 4.1)**

**Catalog No.**

**SAMPLE: 5 preps**

**GF-FE-025: 25 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 Food DNA Extraction Kit** is designed for rapid and efficient purification of genomic DNA from up to 100mg of raw or processed food from plant, animal or mixed origins. This kit uses a specially-treated glass filter membrane 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 proteins and other impurities, additives, preservatives are removed during the subsequent washing steps. High purity genomic DNA is then eluted in water or low salt buffers, ready to use in many routine molecular biology applications such as PCR and restriction enzyme digestion. This kit is suitable for use in GMO testing and has been tested on various raw samples such as soybean, maize and processed foods (soy flour, soymilk, soy sauce, sausage, meat, cereal meal, and cocoa).

## Kit components

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Product Catalog No.	5 Preps SAMPLE	25 Preps GF-FE-025
<b>Components</b>		
GF-1 columns	5	25
Collection tubes	5	25
Food Lysis Buffer ( <b>Buffer FL</b> )	5ml	25ml
Food DNA Binding Buffer ( <b>Buffer FB</b> )	4ml	20ml
Wash Buffer 1 (concentrate)*	1.5ml	7ml
Wash Buffer 2 (concentrate)*	2.4ml	9ml
Elution Buffer	1.5ml	2x 2ml
Proteinase K*	0.11ml	0.55ml
Handbook	1	1

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

The **GF-1 Food 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 Supplied by User**

Absolute Ethanol (>95%)

RNase A (DNase-free, 20mg/ml)

## **Reconstitution of Solutions**

The bottle labeled **Wash Buffer 1** and **Wash Buffer 2** contain concentrated buffers to 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 **5.6ml** of absolute ethanol into the bottle labeled **Wash Buffer 2**.

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

Add **7ml** of absolute ethanol into the bottle labeled **Wash Buffer 1**.

Add **21ml** of absolute ethanol into the bottle labeled **Wash Buffer 2**.

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

## **Storage and Stability**

Store all 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 FB** may exhibit salt precipitation due to cold temperature. If this occurs, simply warm the bottle at 55°C - 65°C with occasional mixing until precipitate is completely dissolved.

## **Chemical Hazard**

**Buffer FB** and **Wash Buffer 1** contain guanidine salts which can be harmful when 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 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 FB**, incubate at 55°C - 65°C with occasional mixing until precipitate is completely dissolved.

Pre-set waterbath to 65°C.

Pre-heat **Elution Buffer** at 65°C.

### 1. Sample preparation and cell lysis

#### a. Solid Sample

Grind the sample into powder form using commercial homogenizer or liquid nitrogen with pestle and mortar.

*Lysis is more efficient when the sample is well homogenized. Homogenization method used depends on the type of sample. In some cases, homogenization is not needed but complete suspension is necessary for efficient lysis for samples such as soy flour and cocoa powder*

Place 100-200mg of ground sample into a clean microcentrifuge tube. Add 700µl **Buffer FL** and 20µl **Proteinase K** into the sample. Mix thoroughly by vortexing to obtain a homogenous solution. Incubate at 65°C for 30 min with occasional mixing during incubation to ensure thorough lysis of the sample.

*Use up to 100mg for hygroscopic samples e.g. flour, dried plant, etc. Users may need to increase the volume of Buffer FL until the sample is semi-fluid like.*

#### b. Liquid sample

Transfer 400µl of sample directly into a clean microcentrifuge tube. Add 300µl **Buffer FL** and 20µl **Proteinase K** into the sample. Mix thoroughly by vortexing to obtain a homogenous solution. Incubate at 65°C for 30 min with occasional mixing during incubation to ensure thorough lysis of the sample.

### 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 10 min.

### 2. Homogenization

Centrifuge at 12,000 x g for 5 min to pellet the cell debris and contaminants. Transfer 450µl of cleared supernatant to a clean microcentrifuge tube. Add 1.5 volumes of **Buffer FB** (~680µl) and mix thoroughly by pulsed-vortexing until a homogenous solution is obtained.

Incubate at 65°C for 10 min.

*When transferring the supernatant, avoid touching the pellet and the layer of contaminants on top of the solutions if any. If the supernatant transferred is less than 450 µl, adjust the volume of Buffer FB to be added proportionately.*

### **3. Addition of ethanol**

Add 350µl of absolute ethanol. Mix immediately and thoroughly by pulsed-vortexing to obtain homogenous solution. Centrifuge at 5,000 x g for 1 min.

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

### **4. Loading to column**

Transfer approximately 650µl of supernatant into a column 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.

### **5. Column washing 1**

Wash the column with 500µl **Wash Buffer 1** and centrifuge at 5,000 x g for 1 min. Discard through.

*Ensure that ethanol has been added into the Wash Buffer 1 before use (refer to Reconstitution of Solutions).*

### **6. 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 the 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).*

*Ensure centrifugation for 3 min to remove ethanol completely.*

### **7. DNA Elution**

Place the column into a clean microcentrifuge tube. Add 50-100µl of preheated **Elution Buffer**, TE buffer or sterile water directly onto column membrane and stand for 2 min. Centrifuge at 5,000 x 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.*

*Note: The recommended amplicon length for PCR analysis is <300bp because DNA in processed food may be degraded to a certain extent.*

## 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:

<b>Problem</b>	<b>Possibility</b>	<b>Suggestions</b>
<b>Low DNA yield</b>	<i>Sample homogenization is not sufficient</i>	<i>Grind the samples into fine powder in liquid nitrogen.</i>
	<i>Insufficient sample lysis</i>	<i>Mix the sample thoroughly with <b>Buffer FL</b> and <b>Proteinase K</b>. Incubation time may be extended up to 2 hours at 65°C.</i>  <i>Reduce the amount of starting material.</i>  <i>If sample used is highly hygroscopic, increase the volume of <b>Buffer FL</b> until the sample is semi-fluid like.</i>
	<i>Addition of ethanol was neglected</i>	<i>Repeat purification with a new sample.</i>
	<i><b>Proteinase K</b> activity is decreased</i>	<i>For long term storage, ensure that <b>Proteinase K</b> is stored at 4°C or -20°C.</i>
	<i><b>Wash Buffer 1</b> and <b>Wash Buffer 2</b> are applied in wrong order</i>	<i>Ensure that <b>Wash Buffer 1</b> is applied before <b>Wash Buffer 2</b>. Repeat purification with a new sample.</i>
	<i><b>Wash Buffer 1</b> and <b>Wash Buffer 2</b> are reconstituted wrongly</i>	<i>Please refer to the 'Reconstitution of Solution'. Repeat purification with a new sample.</i>

<b>Problem</b>	<b>Possibility</b>	<b>Suggestions</b>
<b>Low DNA yield</b>	<i>Column is not dried before addition of <b>Elution Buffer</b></i>	<i>Ensure that column is spun dried at maximum speed for 3 minutes after addition of <b>Wash Buffer 2</b>.</i>
	<i>Elution is not performed properly</i>	<i>Pre-heat <b>Elution Buffer</b> at 65°C -70°C before eluting DNA.</i>  <i>Incubate at room temperature for 2 minutes after addition of <b>Elution Buffer</b>.</i>
		<i>Ensure that <b>Elution Buffer</b> used is a low salt buffer or water with pH range of pH 7.0- 8.5</i>
<b>Low purity (A<sub>260/280</sub>)</b>	<b>Proteinase K</b> activity is decreased	<i>Please refer to problem "Low DNA yield"</i>
	Incomplete sample lysis	<i>Please refer to problem "Low DNA yield"</i>
	<b>Wash Buffer 1</b> is not applied	<i>Ensure that <b>Wash Buffer 1</b> is applied to the column before addition of <b>Wash Buffer 2</b>.</i>
<b>Poor performance of eluted DNA in downstream applications</b>	<i>Eluted DNA contains traces of ethanol</i>	<i>Centrifuge the column at maximum speed for 3 min during second washing of column with <b>Wash Buffer2</b>.</i>
	<i>Eluted DNA contains traces of inhibitors</i>	<i>Ensure that washing steps are carried out with <b>Wash Buffer 1</b> and <b>Wash Buffer2</b></i>
	<i>TE buffer is used to elute DNA. EDTA in TE buffer may inhibit subsequent enzymatic reaction</i>	<i>Use <b>Elution Buffer</b> or water with a pH range of 7.0 - 8.5</i>

