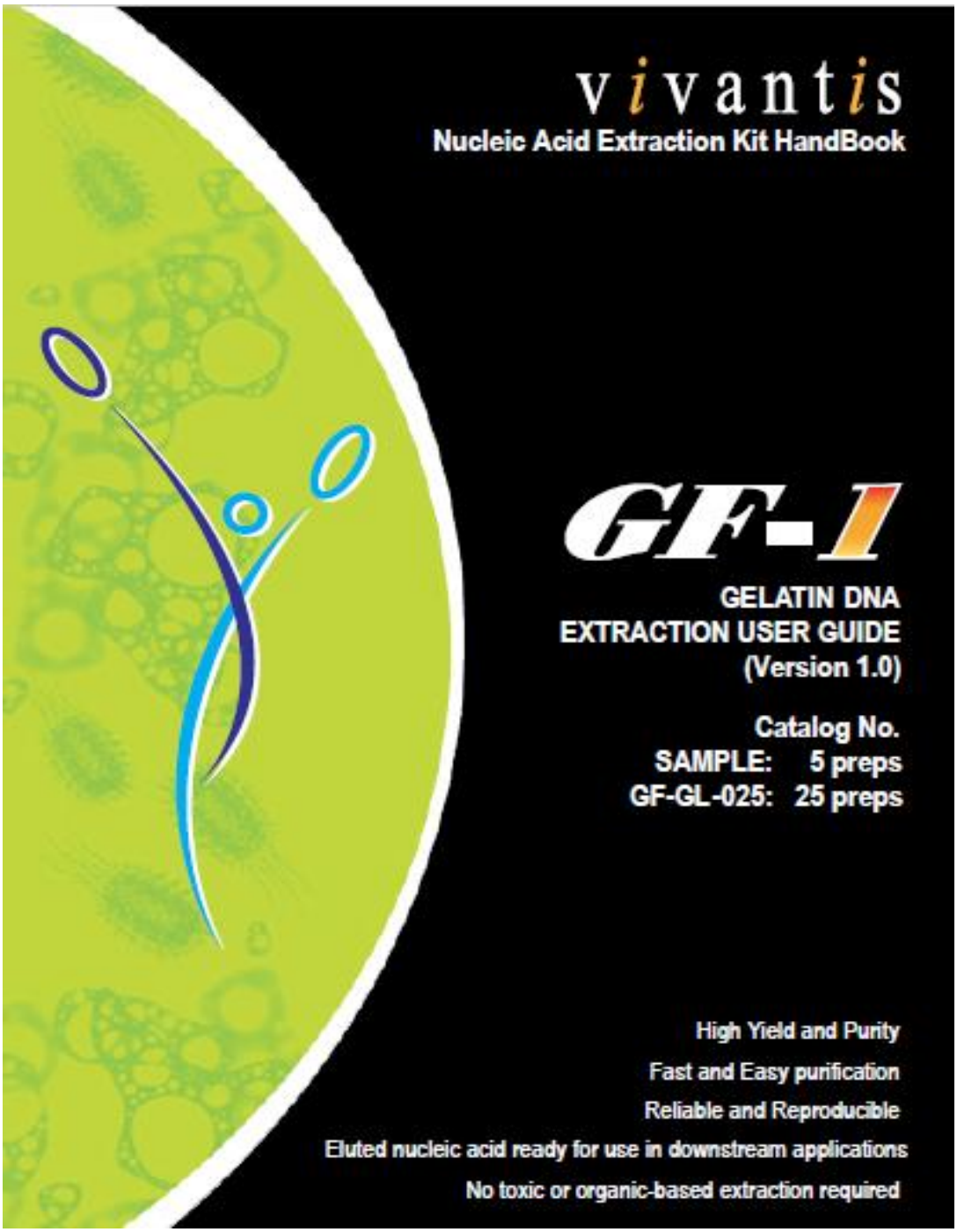
vivantis

Nucleic Acid Extraction Kit HandBook



GF-1

GELATIN DNA EXTRACTION USER GUIDE (Version 1.0)

Catalog No.
SAMPLE: 5 preps
GF-GL-025: 25 preps

High Yield and Purity
Fast and Easy purification
Reliable and Reproducible
Eluted nucleic acid ready for use in downstream applications
No toxic or organic-based extraction required

Introduction

The **GF-1 Gelatin DNA Extraction Kit** is designed for rapid and efficient purification of gelatin from samples such as desserts, marshmallows, yoghurts, soft capsules for supplements and many fat-reduced foods to juices. The purification is based on the usage of detergents and denaturing agents to provide efficient lysis, denaturation of proteins and subsequent release of DNA. Special buffers provided in the kit are optimized for efficiency recovery of highly pure DNA from various types of samples. High purity genomic DNA is then eluted in water or low salt buffer, ready to use in many routine molecular biology applications such as PCR, halal testing and other manipulations.

Kit components

Product	5 Preps	25 Preps
Catalog No.	SAMPLE	GF-GL-025
Components		
Buffer CT	52.5ml	87ml x 3
Buffer IP (concentrate)*	2.3ml	11.5ml
Wash Buffer (concentrate)*	2.1ml	10.5ml
Buffer EB	1.5ml	1.5ml x 2
Buffer GS*	25µl	125µl
Proteinase K*	0.11ml	0.55ml
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* Please refer to **Reconstitution of Solutions** and **Storage and Stability** before using this kit.

The **GF-1 Gelatin 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

Chloroform
Nuclease Free Water

Reconstitution of Solutions

The bottles labeled **Buffer IP** and **Wash Buffer** contain concentrated buffers which must be diluted with Nuclease Free Water before use.

For **SAMPLE (5 preps),**

Add **0.58ml** of Nuclease Free Water into the bottle labeled **Buffer IP**.
Add **0.7ml** of Nuclease Free Water into the bottle labeled **Wash Buffer.**

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

Add **2.9ml** of Nuclease Free Water into the bottle labeled **Buffer IP.**
Add **3.5ml** of Nuclease Free Water into the bottle labeled **Wash Buffer**.

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

Storage and Stability

Store all solutions at 20°C - 30°C.
Store **Buffer GS** and **Proteinase K** at -15°C - -25°C.
Kit components are guaranteed to be stable for 18 months from the date of manufacture.
Buffer CT 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 CT and **Buffer IP** 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
- **Buffer IP** and **Wash Buffer** (concentrate) have to be diluted with Nuclease Free Water before use. Please refer to **Reconstitution of Solutions.**
- If precipitation forms in **Buffer CT**, 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.

I. Sample Lysis

1. For powder form, weight for 2g of samples. For sample other than powder form, cut the sample into smaller pieces and weight for 2g of samples. Add 10ml of **Buffer CT** and 10µl of **Proteinase K** to the sample. Mix thoroughly by vortexing the sample to obtain homogenous solution.

2. Incubate at 65°C for 2 hours in a shaking waterbath or mix occasionally during incubation to ensure thoroughly digestion of the sample.

3. Centrifuge at 4000 x *g* for 10 min. Transfer 800µl of supernatant into new 1.5ml tube.

4. Add 600µl of **Chloroform** and mix the sample solution by vortexing. Centrifuge for 15 min at 10000x *g*.

II. DNA Precipitation

1. Aliquot 600µl of aqueous supernatant into a new 1.5ml tube.

2. Add 2µl of **Buffer GS** and 480µl of **Buffer IP** and mix by vortexing. Leave tube at room temperature 30 min or more for precipitation.
Ensure that Nuclease Free Water has been added into Buffer IP before use (refer to Reconstitution of Solutions).

3. Centrifuge for 15 min at 10000x g to precipitate the DNA. Discard all supernatant carefully by not disturbing the pellet formed at bottom of tube.

III. DNA Washing

1. Add 500µl of **Wash Buffer** and mix through vortexing.

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

2. Centrifuge for 5 min at 10000 x *g*. Discard the supernatant by not disturbing the pellet.
3. Centrifuge again at 10000 x *g* for 1 min. Remove the remaining supernatant carefully.

The remaining supernatant has to be removed, so that the waste residual can be removed completely. The tube can be left at room temperature for 2 min to dry the waste residual completely.

IV. DNA Elution

Add 50-100µl of **Elution Buffer** (preheated at 65°C) directly onto the DNA pellet and dissolve the DNA pellet. Store DNA at 4°C or -20°C.

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	*Samples are too big in pieces*	*For hard or soft pieces of samples, it is advised to cut into smaller piece for efficient lysis.*
	Insufficient nuclease inhibition during sample lysis step	*Ensure that* **Buffer CT** *is mixed homogeneously with the mixture of sample and* **Proteinase K**
	Low quality of **Proteinase K**	*Ensure that the* **Proteinase K** *is aliquoted and can only be thawed not more than once. Please refer to the "***Storage and Stability***".*
	Waste residual is not dried before addition of **Elution Buffer**	*Ensure that waste residual is removed completely and DNA pellet is dried from waste residual.*
Poor performance of eluted DNA in downstream applications	*Eluted DNA contains traces of waste*	*Ensure that the DNA pellet is removed completely from waste residual prior to elution.*
	TE buffer is used to elute DNA. EDTA in TE buffer may inhibit subsequent enzymatic reaction	*Use water with pH range 7.0-8.5.*