vivantis

Nucleic Acid Extraction Kit HandBook

GF-1

FORENSIC DNA EXTRACTION KIT USER GUIDE (Version 2.2)

Catalog No.

GF-FD-005	5preps
GF-FD-025	25preps

Rapid and efficient purification of DNA from traces of biological materials

No organic-based extraction required

Highly pure genomic DNA ready to use for routine molecular biology applications such as restriction enzyme digestion, PCR, Southern blotting, etc.

Introduction

The **GF-1 Forensic DNA Extraction Kit** is designed for rapid and efficient purification of DNA from traces of biological materials such as bloodstains, biological fluids, buccal swab, saliva, semen, hair, feather and nail. This 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 principles of a mini-column spin technology and the use of optimized buffers to ensure that only DNA is isolated while cellular proteins, metabolites, salts and other low molecular weight impurities are removed during subsequent washing steps. 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, restriction enzyme digestion, Southern blotting and other manipulations.

Kit components

Product Catalog No.	5 preps SAMPLE	25 preps GF-FD-025
Components		
GF-1 DNA Binding Columns	5	25
Collection Tubes	15	75
Buffer STL	2ml	10ml
Buffer BL	2ml	10ml
Equilibration Buffer	0.7ml	3.5ml
HB Buffer	3ml	15ml
DNA Wash Buffer*	1.5ml	7.5ml
Elution Buffer	2ml	15ml
OB Protease*	0.15ml	0.75ml
Handbook	1	1

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

The **GF-1 Forensic 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%)
Isopropanal
Phosphate Buffered Saline (PBS)
1M Dithiothreitol (DTT)
RNase A (20mg/ml)
Buffer A (150mM NaCl, 10mM EDTA, pH8.0)
Buffer B (100mM Tris-HCl, pH8.0, 10mM EDTA, 500mM NaCl, 1% SDS, 2% ß-mercaptoethanol)

Reconstitution of Solutions

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

For **SAMPLE (5 preps)**,

Add **6ml** of absolute ethanol into the bottle labeled **DNA Wash Buffer**.

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

Add **30ml** of absolute ethanol into the bottle labeled **DNA Wash Buffer**

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

Storage and Stability

Store all solutions at 20°C - 25°C except **OB Protease**.
Store **OB Protease** at -20°C. We recommend users to store **OB Protease** in small aliquots to avoid repeated freeze-thaw cycles.
Kit components are guaranteed to be stable for 18 months from the date of manufacture.
Buffer BL 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 BL 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.
- **DNA Wash Buffer** (concentrate) has to be diluted with absolute ethanol before use. Please refer to **Reconstitution of Solutions**.
- If precipitation forms in **Buffer BL**, incubate at 55°C - 65°C with occasional mixing until precipitate is completely dissolved.

Pre-set waterbath to 60°C.
Pre-set another waterbath to 55°C.
Pre-heat **Elution Buffer** at 70°C.

I. Sample preparation

A. Dried blood, body fluids and semen spots.

1. Cut or punch out sample spot from filter paper (do not exceed 1cm^2). Cut the filter paper into small pieces and place into a 1.5ml microcentrifuge tube.
2. Add 200µl Buffer **STL** into the tube and mix thoroughly by vortexing. Incubate at 55°C for 15 min with occasional mixing every 2 min.
3. Add 25µl of **OB Protease** and mix thoroughly by vortexing. Incubate at 60°C for 45 min with occasional mixing every 15 min.
4. Add 225µl **Buffer BL** and mix by vortexing. Incubate at 60°C for 10 min.
5. Add 300µl isopropanol and mix thoroughly by vortexing.
6. Proceed to **II. DNA isolation**.

B. Semen

Prepare Buffer A and Buffer B before start. Please refer to **Additional Materials to be Supplied by User**.

1. Add 50-250µl semen sample into 10ml of Buffer A in a glass (Corex) centrifuge tube. Mix thoroughly by vortexing at maximum speed for 10 sec. Centrifuge at 2,500 x *g* for 10 min.
   Frozen sample must be thawed thoroughly before use. Corex tube can prevent attachment of sperm cells on the wall of the tube.

2. Remove the supernatant carefully leaving ~1ml of Buffer A and pellet.

3. Vortex for 10 sec and brief centrifuge at maximum speed to collect any sample adhering on wall of the tube. Transfer all sample to a 1.5ml microcentrifuge tube.

4. Add 0.5ml Buffer A to the Corex tube and vortex for 30 sec. Brief centrifuge at maximum speed to collect any sample adhering on wall of the tube. Transfer all sample to the same 1.5ml microcentrifuge tube.

5. Centrifuge at maximum speed (14,000 x *g*) for 2 min. Remove the supernatant without disturbing the semen pellet.

6. Resuspend the pellet in 200µl of Buffer B. Add 50µl **OB Protease** and mix thoroughly by vortexing. Incubate at 60°C for 2 hours with occasional mixing every 30 min.
   Lysis time may vary depending on the size and density of the source material.

7. Add 250µl **Buffer BL** and mix thoroughly by vortexing.

8. Add 250µl absolute ethanol and mix thoroughly by vortexing.

9. Proceed to **II. DNA isolation**.

C. Buccal swab

1. Scrape the swabs firmly against the inner surface of each cheek 6-7 times.

2. Cut the end of the swab stick containing the sample and place it into a 2ml microcentrifuge tube.

3. Add 550µl **PBS**, 25µl **OB Protease** and 550µl **Buffer BL** to the sample. Mix thoroughly by vortexing. Incubate at 60°C for 30 min with occasional mixing.

4. Add 550µl absolute ethanol and mix thoroughly by vortexing.

5. Proceed to **II. DNA isolation**.

D. Bacteria from biological fluids

1. Put the sample into a clean microcentrifuge tube. Centrifuge at 5,000 x *g* for 10 min to pellet bacteria cells. Remove the supernatant.
2. Resuspend the bacterial pellet in 200µl **Buffer STL**.
3. Add 25µl of **OB Protease** and mix thoroughly by vortexing. Incubate at 60°C for 45 min with occasional mixing every 15 min.
4. Add 225µl **Buffer BL** and mix by vortexing. Incubate at 60°C for 10 min.
5. Add 300µl isopropanol and mix thoroughly by vortexing.
6. Proceed to **II. DNA isolation**.

E. Eye, Nasal, and other swabs

1. Collect the sample in a clean 2ml microcentrifuge tube. Add 1.5ml PBS into the tube and mix thoroughly by vortexing. Incubate at 30°C for 2-3 hours.
2. Centrifuge at 5,000 x *g* for 10 min to pellet bacteria cells. Remove the supernatant.
3. Resuspend the bacterial pellet in 200µl **Buffer STL**.
4. Add 25µl of **OB Protease** and mix thoroughly by vortexing. Incubate at 60°C for 45 min with occasional mixing every 15 min.
5. Add 225µl **Buffer BL** and mix by vortexing. Incubate at 60°C for 10 min.
6. Add 300µl isopropanol and mix thoroughly by vortexing.
7. Proceed to **II. DNA isolation**.

F. Saliva

1. Collect 1.5ml saliva and put into a clean 15ml centrifuge tube containing 6ml PBS. Mix thoroughly by vortexing.
2. Centrifuge at 2,000 x *g* for 5 min. Discard the supernatant and resuspend the pellet in 180µl PBS.
3. Transfer the samples into a clean 1.5ml microcentrifuge tube.

   Optional: Removal of RNA
   If RNA-free DNA is required, add 20ml RNase A (DNase-free, 20mg/ml) to the sample.
4. Add 25µl **OB Protease** and 200µl **Buffer BL** to the sample. Mix thoroughly by vortexing for 30 sec. Incubate at 60°C for 15 min with occasional mixing every 5 min.
5. Add 200µl absolute ethanol and mix thoroughly by vortexing.
6. Proceed to **II. DNA isolation**.

G. Hair, nails and feathers

1a. Cut hair fragment into small pieces (0.5 – 1cm), with the root included (maximum 20mg).

1b. Cut nails into small pieces of size less than $2mm^2$ (maximum 20mg).

1c. Cut primary feather into small pieces (0.5-1cm). For large bird, secondary tail or breast feather can be used.

2. Place the sample into a 1.5ml microcentrifuge tube. Add 250µl **Buffer STL**, 25µl **OB Protease** and 20µl 1M DTT into the sample. Mix thoroughly by vortexing. Incubate at 60°C for 30 min with occasional mixing.

3. Add 250µl **Buffer BL** and mix thoroughly by vortexing.

4. Add 250µl absolute ethanol and mix thoroughly by vortexing

5. Proceed to **II. DNA isolation**.

II. DNA isolation

1. Transfer 600µl of sample to a column assembled to a clean collection tube (provided). Centrifuge at 8,000 x *g* for 1 min. Discard flow through.

   Repeat for the remaining sample if any. Discard flow through and the collection tube. Insert the column to a new collection tube.

2. Add 500µl **HB Buffer** and centrifuge at 8,000 x *g* for 1 min. Discard flow through.

3. Wash the column with 750µl **Wash Buffer** and centrifuge at 8,000 x *g* for 1 min. Discard flow through and collection tube.
   Ensure that ethanol has been added onto the Wash Buffer before use (refer to Reconstitution of Solutions).

4. Insert the column to a new collection tube. Wash the column again with 750µl **DNA Wash Buffer** and centrifuge at 8,000 x *g* for 1 min. Discard flow through. Centrifuge the column at maximum speed (>13,000 x *g*) for 2 min to remove residual ethanol.

5. Place the column into a clean microcentrifuge tube. Add 50-100µl **Elution Buffer** (preheated at 70°C) directly onto the membrane and stand for 3 min. Centrifuge at 8,000 x *g* for 1 min. Store DNA at 4°C or -20°C.
   Ensure that Elution Buffer is dispensed directly onto the center of the membrane for completer 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 the RNA may occur. If problems arise, please refer to the following:

Problem	Possibility	Suggestions
Low DNA yield	*Incomplete sample lysis*	*Extend incubation time of lysis with* **Buffer STL** *and* **OB Protease** *by 10 min.*
		Ensure that sample is cut into small pieces.
		Ensure that the amount of **Buffer BL** *is added correctly. Mix* **Buffer BL** *with sample thoroughly by vortexing.*
	Addition of ethanol was neglected	*Ensure that ethanol is added prior to sample loading to column. Repeat purification with new sample.*
	Column clogged	*Please refer to the suggestions for "Incomplete sample lysis".*
	OB Protease *activity is decreased*	*Prepare* **OB Protease** *in small aliquots to avoid repeated freeze-thaw cycles. Ensure that* **OB Protease** *is stored at -20°C.*
	DNA Wash Buffer *is reconstituted wrongly*	*Please refer to "Reconstitution of Solutions". Repeat purification with a new sample.*
	Low elution efficiency	*Pre-heat* **Elution Buffer** *to 70°C before eluting DNA.*
		Incubate column at room temperature for 3 min after addition of **Elution Buffer**.

Problem	Possibility	Suggestions
Low Purity (A260/280)	**OB Protease** *activity is decreased*	*Please refer to "Low DNA yield".*
	Incomplete sample lysis	*Please refer to "Low DNA yield".*
Poor performance of eluted DNA in downstream applications	*Eluted DNA contains traces of ethanol*	*Centrifuge the column at maximum speed (>13,000 x g) for 2 min to remove residual ethanol.*
	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*