RUO

REF	G2MBR4-0583	50 Tests
	G2MBR4-0584	250 Tests

SPINXT

Forensic DNA Extraction Kit

Intended Use

The SpiNXT Forensic DNA Extraction Kit is intended for molecular biology applications. This kit is designed for isolation of genomic DNA from Mammalian/Human cells & tissues, human whole blood, dried blood spots, cell culture pellet, swabs, buccal cells, buffy coat, plasma, serum, lymphocytes, challenging body fluids, vaginal and semen stains, bone, tooth, hair, chewing gum, cigarette butts and other forensic samples.

Intended User

The assay is intended to be performed by a laboratory professional in research laboratory.

Summary

SpiNXT Forensic DNA Extraction Kit allows rapid and efficient preparation of highly pure genomic DNA. Purification requires no phenol/chloroform extraction or alcohol precipitation, involves minimal handling and simple centrifugation processing which completely removes contaminants and enzyme inhibitors, such as proteins and divalent cations. The kit is designed to efficiently isolate genomic DNA from mammalian/ human cells, tissues, fresh or frozen whole blood or blood which has been treated with citrate, heparin or EDTA, dried blood spots, cell culture pellet, swabs, buccal cells, buffy coat, plasma, serum, lymphocytes, challenging body fluids, vaginal and semen stains, bone, tooth, hair, chewing gum, cigarette butts and other forensic samples. (*Additionally, for maximum and efficient yields from DBS samples - an alternative DBS Lysis Buffer is recommended).

Equipment and Reagents Required but Not Provided

- Water bath or Heat block
- Micropipettes (Adjustable)
- Disposable barrier (Filter) pipette tips
- 1.5/2 ml microcentrifuge tubes
- Table top microcentrifuge
- Molecular biology grade ethanol (96-100 %)
- Personal protection equipment (lab coat, goggles etc.)
- Disposable powder free gloves
- Mortar and Pestle
- DBS Lysis Buffer (*Can be supplied by Genes 2Me on request)
- Buffer 1X PBS

Storage and Stability

- The kit has a shelf life of 18 months from the date of manufacture.
- The test kit is stable until the expiration date marked on the kit box and/or the packaging of individual components when stored at room temperature (15-25 °C).
- If precipitate forms in any of the reagents of the kit, warm at 55 °C to dissolve.
- After reconstitution, Proteinase K needs to be stored at -20 °C.

Instructions Before Use

- Switch on the water bath/heat, set the temperature controls to the desired temperature and wait until the thermostat reaches the range (56 & 70 °C; 85 °C for DBS samples) before starting of the experiment.
- Use preheated Buffer AE (50 °C) for efficient DNA yield.
- Use sterile 1.5/2 ml microcentrifuge tubes.

Reagents Provided

Table 1a. (For 50 Tests)

Kit Contents	Kit Content Code	Kit Content Quantity G2MBR4-0583
Buffer LB	G2MBR3-1933-1	15 ml
Buffer BW1	G2MBR3-1934-1	20 ml
Buffer BW2	G2MBR3-1935-1	20 ml
Proteinase K	G2MBR3-1936-1	30 mg
Protease Dissolve Buffer	G2MBR3-1937-1	4 ml
Buffer AE	G2MBR3-1938-1	10 ml
Buffer LBTC	G2MBR3-1939-1	15 ml
Buffer TBLB	G2MBR3-1940-1	25 ml

Consumables Provided

Table 1b. (For 50 Tests)

Kit Contents	Kit Content Quantity G2MBR4-0583
Mini Column	1 X 50 Nos.
Collection Tube	1 X 50 Nos.

Reagents Provided

Table 2a. (For 250 Tests)

Kit Contents	Kit Content Code	Kit Content Quantity G2MBR4-0584
Buffer LB	G2MBR3-1933-2	55 ml
Buffer BW1	G2MBR3-1934-2	90 ml
Buffer BW2	G2MBR3-1935-2	2 X 45 ml
Proteinase K	G2MBR3-1936-2	120 mg
Protease Dissolve Buffer	G2MBR3-1937-2	10 ml
Buffer AE	G2MBR3-1938-2	30 ml
Buffer LBTC	G2MBR3-1939-2	55 ml
Buffer TBLB	G2MBR3-1940-2	110 ml

Consumables Provided

Table 2b. (For 250 Tests)

Kit Contents	Kit Content Quantity G2MBR4-0584
Mini Column	2 X 125 Nos.
Collection Tube	2 X 125 Nos.

- Before using for the first time, add the appropriate volume of molecular biology grade ethanol (96–100 %) as indicated on the bottle and shake thoroughly. Buffer BW1 and Buffer BW2 are stable for at least one year after the addition of ethanol, when stored at room temperature (15–25 °C) in closed condition.
- Add Proteinase K into absolute amount of Protease Dissolve Buffer, as mentioned on the label, and store at -20 °C.

Important Notes

- Sample collection and storage shall be performed according to the recommended procedure.
- Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA yield.
- Use of poor-quality starting material may affect yield of purified DNA.

Protocol

A. DNA Purification from Mammalian/Human Cells and Tissues

1). Cut ~25 mg of tissue sample into smaller pieces or grind using Mortar and Pestle for efficient lysis. Transfer it into a 1.5/2 ml microcentrifuge tube and 180 µl Buffer LBTC is added into it along with 20 µl Proteinase K.

2). Incubate the mixture at 56 °C for 20 min which can be extended up to 1-3 hours depending upon the tissue sample or till the lysis of the cells.

3). 200 µl Buffer LB is added to the solution followed by incubation at 70 °C for 10-15 min.

4). Further add 200 µl of chilled (96-100 %) ethanol into the solution.

5). Pipette the lysate mixture and transfe into the Mini Column, centrifuge at 6,000 x*g* for 1 min. Discard collection tube with flow-through.

6). Place the Mini Column back into a fresh Collection Tube (2 ml) and add 700 µl Buffer BW1. Centrifuge for 1 min at 10,000xg. Discard the flow-through.

7). Place the Mini Column into the Collection Tube (2 ml) and add 700 µl Buffer BW2. Centrifuge for 1 min at 10,000 xg. Discard the flow- through.

8). Repeat step 7.

9). Dry spin: Centrifuge the tube one more time at full speed or 20,000 xg for 2 mins.

10). Place the column into a fresh 1.5/2 ml microcentrifuge tube and apply 50 °C prewarmed 30-50 µl Buffer AE directly to the center of the silica membrane. Incubate at room temperature (18-25 °C) for 3-5 min. Centrifuge at 6,000 xg for 1 min.

10). Store the purified DNA at -20 °C for long-term storage.

B. DNA Purification from Dried Blood/Vaginal/Semen spots

1). Cut the section of filter which contains the dried sample and place it in a sterile 1.5/2 ml microcentrifuge tube.

2). Add 200 µl of Buffer 1XPBS and incubate for 5-10 min at room temperature.

3). Add 200 µl Buffer LB and incubate at 85 ºC for 20 min. For more efficient yields, DBS Lysis Buffer is recommended.

4). Following efficient lysis add 200 µl Buffer LB with additional 20 µl Proteinase K and incubate it at 56 ºC for 20 min. Centrifuge it at 10,000 xg for 2 min.

5). Collect the supernatant into a new 1.5/2 ml microcentrifuge and add 0.5 ml of absolute ethanol (96-100 %). Transfer whole of the lysate onto a mini column and centrifuge it at 10,000 xg for 1 min. Discard the flow-through and place it back into the collection tube.

6). Proceed with the step 6 of DNA purification for mamalian/human cells and tissues.

C. DNA Purification from Buccal Swabs

1). To collect a sample, scrape the swab 5-6 times against the inside cheek.

2). Swirl the swab for 30-60 sec in 1-2 ml of Buffer 1XPBS.

3). 200 µl of sample is transferred into a 1.5/2 ml microcentrifuge tube.

4). Add 20 µl Proteinase K and 200 µl Buffer LB. Mix by 10 sec short vortexing and incubate at 56 °C for 10 min in water bath.

5). Proceed with the step 4 of DNA purification for mammalian/human cells and tissues.

D. DNA Purification from Cultured Cells

1). Centrifuge the appropriate number of cells (5 x 10^6) for 10 min at 5000 xg. Discard the supernatant in such a way that 20 µl of supernatant remain in it and then resuspend the pellet in 180 µl Buffer LBTC.

2). 200 µl of sample is transferred into a 1.5/2 ml microcentrifuge tube.

3). Add 20 µl Proteinase K and 200 µl Buffer LB. Mix by 10 sec short vortexing and incubate at 56 °C for 10 min in water bath.

4). Proceed with the step 4 of DNA purification for mammalian/human cells and tissues.

E. DNA Purification from Whole Blood

1). 200 µl of sample is transferred into a 1.5/2 ml microcentrifuge tube. Add 20 µl Proteinase K and 200 µl Buffer LB. Mix by 10 sec short vortexing and incubate at 56 °C for 20 min in water bath.

2). Proceed with the step 4 of DNA purification for mammalian/human cells and tissues.

F). DNA Purification from Buffy Coat/Lymphocytes

1). Buffy coat is a leukocyte-enriched portion of whole blood and contains approximately 5-10 times more DNA than an equivalent volume of whole blood. Prepare the buffy coat by centrifuging whole blood at 1000 x*g* for 10 min at room temperature. After centrifugation, three different fractions are distinguished: the upper clear layer contains plasma; the intermediate buffy coat layer containing concentrated leukocytes, and the bottom layer containing concentrated erythrocytes.

2). Collect approximately 200 µl of intermediate layer using micropipette into a fresh and sterile (If necessary, adjust the volume to 200 µl with Buffer 1XPBS) 1.5/2 ml microcentrifuge tube.

3). Add 20 µl Proteinase K and 200 µl Buffer LB. Mix by 10 sec short vortexing and incubate at 56 °C for 10 min in water bath.

4). Proceed with the step 4 of DNA purification for mammalian cells and tissues.

G. DNA Purification from Plasma/Serum

1). Centrifuge 1.5/2 ml of whole blood at 3000 x*g* for 10 min at room temperature. Three layers will be visible.

2). Collect approximately 200 µl of upper layer containing plasma/serum carefully into a fresh and sterile 1.5/2 ml microcentrifuge tube.

3). Add 20 µl Proteinase K and 200 µl Buffer LB. Mix by 10 sec short vortexing and incubate at 56 °C for 10 min in water bath.

4). Proceed with the step 4 of DNA purification for mammalian/human cells and tissues.

H. DNA Purification from Cigarette Butts/Chewing Gums

1). Cigarette butts: Cut ~ 8 mm from the end of the cigarette butt, and place end down in the tube. Add 200 µl Buffer LB along with 20 µ l Proteinase K and incubate the mixture at 56 ºC for 20 min followed by 1 min vortexing.

2). Collect the supernatant in a 1.5/2 ml microcentrifuge tube.

3). Proceed with the step 4 of DNA purification for mammalian/human cells and tissues.

I. DNA Purification from Hair

1). Process the root portion of the human hair and cut it into smaller pieces.

2). Transfer it into a 1.5/2 ml microcentrifuge tube and 180 µl Buffer LBTC is added into it along with 20 µl Proteinase K.

3). Proceed with the step 2 of DNA purification for mammalian/human cells and tissues.

J. DNA Purification from Bone/Tooth

1). Grind the bone and tooth sample into fine powder by mixing it with liquid nitrogen in a mortar and pestle.

2). Transfer the crushed powder (upto 20 mg) into a fresh 1.5/2 ml microcentrifuge tube and add 100 µl Proteinase K into it.

3). Mix by vortexing for 15-30 sec.

4). Further, add 400 µl Buffer TBLB into the solution and invert mix for 3-4 times.

5). Incubate the solution for about 2.5 hours at 56 ºC in a Thermo mixer at 1200 rpm (Preferable time is about 2.5 hours but can be extended for optimal digestion).

6). Centrifuge it for 5 min at 5000 x*g*.

7). Discard the pellet and carefully aspirate the supernatant to a fresh 1.5/2 ml microcentrifuge tube.

8). Add 500 µl Buffer LB to the micro centrifuge tube and invert mix for about 3-4 times and then add 450 µl absolute ethanol (96-100 %).

9). Invert mix the microcentrifuge tube again for about 3 - 4 times and let it settle for 1 min at room temperature.

10). Pipette the mixture on the Mini Column, centrifuge at 10,000 x*g* for 1 min. Discard collection tube with flow-through.

11). Proceed with the step 6 of DNA purification for mamalian/human cells and tissues.

Troubleshooting Guide

A. Poor yield / low sensitivity

1) Incomplete sample lysis

- Reduce the amount of starting material used.
- Ensure to add Proteinase K during lysis.
- For tissues, cut the tissue into smaller pieces, if it is fragile and ensure the tissue is completely immersed in the LBTC Buffer to obtain optimal lysis.
- If incomplete lysis is observed, increase the digestion time as mentioned in the lysis protocol or amount of Proteinase K used for lysis.

B. Clogged columns

1) Excess test sample material inputs

- Do not use more sample material than recommended (~25 mg for most tissue types).
- If insoluble material like bones or hair remains in the lysate, spin down the debris and transfer the clear supernatant to a new microcentrifuge tube before adding Buffer LB to the sample. Remove any particulate or viscous material by centrifugation and ensure that the lysate is clear and free of any debris/undigested visible particles prior to loading on to the spin column.

C. Poor quality of sample material

- Process the sample immediately after collection or store the samples at optimal temperature. The yield and quality of DNA isolated is dependent on the type and age of the starting material.

D. No ethanol reconstitution into BW1 and BW2

- Make sure to add 96–100 % ethanol to BW1 and BW2 as indicated on the label.

E. Incorrect elution conditions

- Add Buffer AE and perform incubation for 3-5 min before centrifugation.

F. DNA is sheared or degraded

- Avoid repeated freezing and thawing of samples to prevent any DNA damage. Maintain a sterile environ-ment while working to avoid any contamination from DNases.

G. Dark colored elute or discolored membrane (mammalian tissue, mouse tails, or blood samples only)

Pigments from tissues or heme from blood bind to the silica matrix and co-elute with DNA

- Make sure to add (96-100 %) ethanol to the lysate prior to loading the lysate on to the mini column. The etha-nol prevents the pigments from sticking on the silica matrix.
- Perform centrifugation of the lysate at a higher speed and longer time prior to loading the lysate on the column.
- If the problem persists, perform an additional wash step.

H. Inhibition of downstream enzymatic reactions

- **Presence of salt in purified DNA**
- Ensure to follow the correct order of Wash Buffers for washing.
- Always wash with Buffer BW1 followed by Buffer BW2 with an additional step of washing of column with 80 % ethanol.

Presence of ethanol in purified DNA

- To remove Buffer BW2 from spin columns, discard the flow-through. Place the spin column into the collection tube for 2–3 min to completely dry the column.

Safety and Precautions

- **Chemical Handling:** Reagent cartridges/plates contain guanidine hydrochloride/guanidine thiocyanate, which may react with bleach to form highly reactive compounds. In case of spillage, clean with laboratory detergent and water.
- **Biological Samples:** Tissues, body fluids, infectious agents, and blood may carry infectious diseases. Ensure all laboratory personnel are familiar with general safety guidelines for chemical usage, storage, and waste disposal. Refer to relevant Safety Data Sheets (SDS) for specific precautions.
- **Personal Protective Equipment (PPE):** Wear appropriate attire, including lab coats, gloves, goggles, and closed-toe shoes to protect against spills, splashes, and inhalation.
- **Ventilation:** Work in well-ventilated areas or use fume hoods to minimize exposure to harmful vapors or inhalation of chemicals. Handle the chemical waste in designated fume hoods.
- **Storage:** Store the chemicals correctly in designated areas, adhering to guidelines for temperature, compatibility, and segregation.
- **Labeling:** Ensure all the containers are clearly labeled with the chemical name, concentration, and hazard warnings to prevent accidents or confusion.
- **Handling:** Employ proper techniques, while handling chemicals, pour chemicals carefully and steadily to avoid spills, splashes, and excessive vapor release. Keep chemical containers closed while not in use.
- **Emergency Equipment:** Familiarize yourself with the location and proper use of safety showers, eyewash stations, fire extinguishers, and spill kits for swift response to emergencies.
- **Training:** All individuals must undergo comprehensive training on chemical handling, emergency procedures, and the use of safety equipment as per regulatory and institutional requirements before handling potentially biohazardous materials.

Symbols for Use in the Labeling	
Symbols	**Definition**
	KEEP AWAY FROM SUNLIGHT
	TEMPERATURE LIMIT
	RESEARCH USE ONLY
	UPWARD
	CONSULT INSTRUCTIONS FOR USE
	BATCH CODE
	CATALOGUE NUMBER
	USE BY DATE
	DATE OF MANUFACTURE
	MANUFACTURER
	CONTAINS SUFFICIENT FOR <n> TESTS
	CAUTION
	DO NOT USE IF PACKAGE IS DAMAGED

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