RUO

Cat. No.	G2MBR4-0579	50 Tests
	G2MBR4-0580	250 Tests

SPINXT Blood DNA Extraction Kit

Intended Use

SpiNXT Blood DNA Extraction Kit is intended for isolation and purification of genomic DNA from whole blood, serum, plasma, buffy coat, lymphocytes samples. SpiNXT Blood DNA Extraction Kit utilizes silica column based technology and can be processed either manually or automated on most open-ended platforms such as silica column processors. SpiNXT Blood DNA Extraction Kit for professional, laboratory trained personnel use only.

Intended User

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

Test Principle

SpiNXT Blood DNA Extraction Kit provides a simple and convenient way to isolate pure genomic DNA from fresh or frozen whole blood or blood which has been treated with citrate, heparin or EDTA and blood components such as lymphocytes, buffy coat, plasma and serum. The extracted DNA can be used for clinical *in vitro* detection and is designed for rapid and efficient purification of high quality genomic DNA. The kit combines the features and advantages of silica binding with a micro spin format, and eliminates the need of hazardous compounds such as phenol/chloroform for extraction or alcohol precipitation and involves minimum handling. This product is suitable for rapid isolation of DNA from ~200 µl blood.

Summary

SpiNXT Blood DNA Extraction kit provides a simple and convenient way to isolate pure genomic DNA from fresh or frozen whole blood or blood which has been treated with citrate, heparin or EDTA and blood components such as lymphocytes, buffy coat, plasma and serum. The extracted DNA can be used for clinical *in vitro* detection and is designed for rapid and efficient purification of high quality genomic DNA. The kit combines the features and advantages of silica binding with a micro spin format, and eliminates the need of hazardous compounds such as phenol/chloroform for extraction or alcohol precipitation and involves minimum handling. This product is suitable for rapid isolation of DNA from ~200 µl blood.

Storage, Operating Conditions and Stability

This product can be stored at room temperature (15-25 °C) for 18 months. If precipitate forms in any of the reagents of the kit, warm at 55 ºC to dissolve. After dissolving, Proteinase K shall be stored at -20 °C.

***RNaseA (10 mg/ml) needs to be stored at 2-8 °C.**

Reagents Provided

Table 1a. (For 50 Tests)

Reagents Provided	Kit Content Code (50T)	Kit Content Qty G2MBR4-0579
Buffer LB	G2MBR3-1925-1	1 X 25 ml
Buffer BW1	G2MBR3-1926-1	1 X 16 ml
Buffer BW2	G2MBR3-1927-1	1 X 15 ml
Binding Buffer	G2MBR3-1928-1	1 X 12 ml
Proteinase K	G2MBR3-1929-1	1 X 20 mg
Protease Dissolve Buffer	G2MBR3-1930-1	1 X 2 ml
Buffer AE	G2MBR3-1931-1	1 X 3 ml
RNaseA	G2MBR3-1932-1	1 X 250 µl

Consumables (50T)
Table 1b. (For 50 Tests)

Reagents Provided	Kit Content Qty G2MBR4-0579
Mini Column	1 X 50 Nos.
Collection Tube	1 X 50 Nos.

Reagents Provided
Table 2a. (For 250 Tests)

Reagents Provided	Kit Content Code (250T)	Kit Content Qty G2MBR4-0580
Buffer LB	G2MBR3-1925-2	1 X 110 ml
Buffer BW1	G2MBR3-1926-2	1 X 80 ml
Buffer BW2	G2MBR3-1927-2	2 X 30 ml
Binding Buffer	G2MBR3-1928-2	1 X 55 ml
Proteinase K	G2MBR3-1929-2	1 X 100 mg
Protease Dissolve Buffer	G2MBR3-1930-2	1 X 6 ml
Buffer AE	G2MBR3-1931-2	1 X 12 ml
RNaseA	G2MBR3-1932-2	1 X 1.25 ml

Consumables (250T)
Table 2b. (For 250 Tests)

Reagents Provided	Kit Content Qty G2MBR4-0580
Mini Column	2 X 125 Nos.
Collection Tube	2 X 125 Nos.

Materials Required but Not Provided

- Water bath or Heating block
- Table top microcentrifuge
- Vortexer
- Disposable barrier (Filter) pipette tips
- Molecular biology grade ethanol (96-100%)
- Micropipettes (Adjustable)
- Personal protective equipment (Aprons, disposable gloves, goggles etc).
- 1.5 ml microcentrifuge tubes (RNase and DNase free)

⚠ Instructions Before Use

- Preheat a water bath or heating block to 56 °C.
- Use preheated Buffer AE (50 °C).
- Buffer BW1 and Buffer BW2 are supplied as concentrates. 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 18 months after the addition of ethanol, when stored closed at room temperature (15-25 °C).
- Add Protease Dissolve Buffer into absolute amount of Proteinase K, as mentioned on the label and store it at -20 °C.

Procotol

A. DNA Purification from Whole Blood

1) Transfer 200 µl Binding Buffer, 200 µl sample and 400 µl Buffer LB to a clean 1.5 ml microcentrifuge tube.
2) Add 20 µl Proteinase K along with 5 µl RNaseA. Mix for 10 sec by short vortexing and incubate at 56 °C for 10-15 min in water bath/Heating block .
3) Add 400 µl volume of 100 % ethanol mix by 10 sec short vortexing.
4) Pipette the mixture (700 µl) onto the gDNA Mini Column. Centrifuge at 10,000 x*g* for 1 min. Discard the Collection Tube with flow-through (Repeat the process until whole solution gets transferred into the Mini Column).
5) Place the gDNA Mini Column into a fresh Collection Tube (2 ml) and add 500 µl Buffer BW1. Centrifuge 1 min at 10,000 x*g*. Discard the flow-through.
6) Place the gDNA Mini Column back into the collection tube and add 500 µl Buffer BW2. Centrifuge for 1 min at 10,000 x*g*. Discard the flow-through.
7) Repeat step 6.
8) Dry spin: Centrifuge the tube one more time at full speed or 20,000 x*g* for 2 min.
9) Place the column into a fresh 1.5 ml microcentrifuge tube and apply 50 °C pre-warmed 40 µl Buffer AE directly to the center of the silica membrane. Incubate at room temperature (18-25 °C) for 2-3 min. Centrifuge at 6,000 x*g* for 1 min.
10) Store the isolated DNA at -20 ºC for long term storage.

B. DNA purification from Buffy Coat/Lymphocytes

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.

1) Collect approximately 200 µl of intermediate layer (if necessary, adjust the volume to 200 µl with 1X PBS) using micropipette into a fresh and sterile 1.5 ml microcentrifuge tube.
2) Proceed with step 1 of DNA purification from whole blood.

C. DNA Purification from Plasma/Serum

1) Centrifuge 1.5 ml of whole blood at 1000 xg for 10 min at room temperature. Three layers will be visible.
2) Collect approximately 200 µl of upper layer plasma/serum containin carefully into a fresh and sterile 1.5 ml microcentrifuge tube.
3) Proceed with step 1 of DNA purification from whole blood.

Troubleshooting guide

A. Poor or low yield of nucleic acid

- **Sample is older:** The yield of the DNA depends upon sample quality, type and volume. Much older samples allow lysis to occur more readily which eventually leads to degradation of DNA.
- **Elution is incomplete/ Elute contains residual ethanol from the Wash Buffers:** In order to remove ethanol completely from the final wash with Wash Buffers. Spin down the tubes for longer time to dry the column completely.
- **Ethanol was not added to wash buffer concentrate:** Check whether ethanol is added to wash buffer concentrate as per the instructions on the label before using.

B. Spin column is clogged

- **Large sample volume:** High cell number in the intial sample may lead to inefficient lysis, eventually lead to the spin column clogging. Use a much lesser quantity of sample. Clogging can be alleviated by centrifuging the column for a longer time period until whole of the lysate passes through or by increasing the *g* force.

C. DNA is sheared or degraded

- **DNA was handled improperly:** Use sterile, disposable plastic ware, glassware and autoclavable pipettes reserved specifically for DNA work to avoid contamination from shared equipments. Pipetting steps should be taken care of. Change the gloves frequently whenever required.

Limitations

- **Sample Variability:** Different body fluids may contain varying amounts of DNA, leading to differences in extraction efficiency and yield. Each sample type requires specific preprocessing and the use of appropriate buffers and protocols. Failure to preprocess samples adequately may result in reduced efficiency or failure of DNA extraction due to potential inhibitors present in certain body fluids.
- **Cross-Contamination:** Improper handling or inadequate cleaning of equipment may lead to cross-contamination between samples, compromising the reliability of the extracted DNA. Maintaining a contamination free environment and following proper cleaning protocols are essential to minimize the risk of cross-contamination.

Safety and Precautions

- **Chemical Handling:** Reagents contain guanidine hydrochloride/guanidine thiocyanate, which can react with bleach to form highly reactive compounds. In case of spillage, clean with laboratory detergent and water.
- **Biological Samples:** 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 chemical waste in designated fume hoods.
- **Storage:** Store chemicals correctly in designated areas, adhering to guidelines for temperature, compatibility, and segregation.
- **Labeling:** Ensure all containers are clearly labeled with the chemical name, concentration, and hazard warnings to prevent accidents or confusion.
- **Handling:** Employ proper techniques when handling chemicals, such as pouring slowly, avoiding splashing, and refraining from pipetting by mouth. Keep chemical containers closed when 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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