CE IVD

REF G2M810013

Cell free DNA Extraction Kit

S. No.	Product Name	SKU Code	Pack Size
1.	SpiNXT Cell free DNA Extraction Kit	G2M810013-50T	50T
2.	SpiNXT Cell free DNA Extraction Kit	G2M810013-250T	250T

Intended Use

SpiNXT Cell free DNA Extraction Kit is an *in-vitro* diagnostic test kit, intended for isolation and purification of cell free DNA from serum & plasma samples. SpiNXT Cell free 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 cell free DNA Extraction Kit for professional, laboratory trained personnel use only.

Intended User

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

Test Principle

This product is based on silica gel purification. The sample is lysed and digested with the lysate and protease, DNA is released into the lysate. Transfer the lysate to an adsorption plate and filter column. Nucleic acid is adsorbed on the membrane, while protein is not adsorbed and is removed with filtration. After washing off the proteins and other impurities, nucleic acid was is finally eluted with low-salt buffer (10 mM Tris, pH 8.0).

Summary

Significant advancements have proven that utilizing circulating Cell free DNA (ccf-DNA) can serve as biomarker and provides real time mutational information that can be used to diagnose/ prognosis & monitor the therapy for several cancer types and autoimmune diseases. Also, it can serve as a potential biomarker for certain cancers as well as fetal DNA in maternal blood. Additionally, cell free circulating DNA is widely being used as a non-invasive method for prenatal screening together with cell free detection of fetal chromosomal abnormalities. The SpiNXT Cell free DNA Extraction Kit enables efficient purification of these circulating nucleic acids from human plasma, or serum. Samples can be rather fresh or frozen (provided that it has not been freeze-thawed more than once).

Storage, Operating Conditions and Stability

- This kit shall be stored at room temperature (15-25 °C) up to 18 months.
- If precipitate forms in any of the reagents of the kit, warm at 55 °C to dissolve.

Reagents Provided

Table 1a. (For 50 Tests)

Kit Contents	Kit Content Code	Kit Content Quantity G2M810013
Binding Buffer	G2MA3-2431-2	1 X 50 ml
Buffer CLB-3	G2MA3-2432-3	1 X 60 ml
Buffer CW1	G2MA3-2433-1	1 X 22 ml
Buffer CW2	G2MA3-2434-1	1 X 10 ml
Proteinase K	G2MA3-2435-2	1 X 120 mg
Protease Dissolve Buffer	G2MA3-2436-3	1 X 10 ml
Carrier RNA	G2MA3-2437-2	1 X 110 µg
Buffer AE	G2MA3-2438-4	1 X 10 ml

Consumables Provided

Table 1b. (For 50 Tests)

Kit Contents	Kit Content Quantity G2M810013
Mini Column	1 X 50 Nos.
Collection Tubes	1 X 50 Nos.

Table 2a. (For 250 Tests)

Kit Contents	Kit Content Code	Kit Content Quantity G2M810013
Binding Buffer	G2MA3-2431-1	1 X 250 ml
Buffer CLB-3	G2MA3-2432-1	1 X 300 ml
Buffer CW1	G2MA3-2433-3	1 X 88 ml
Buffer CW2	G2MA3-2434-2	1 X 50 ml
Proteinase K	G2MA3-2435-1	1 X 540 mg
Protease Dissolve Buffer	G2MA3-2436-1	1 X 30 ml
Carrier RNA	G2MA3-2437-3	1 X 310 µg
Buffer AE	G2MA3-2438-3	1 X 30 ml

Consumables Provided

Table 2b. (For 250 Tests)

Kit Contents	Kit Content Quantity G2M810013
Mini Column	2 X 125 Nos.
Collection Tubes	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 and 5 ml microcentrifuge tubes (RNase and DNase free)

Instructions Before Use

- Buffer CW1, Buffer CW2 and Buffer CLB-3 are supplied as concentrate. Before using for the first time, add the appropriate volume of molecular biology grade ethanol (96-100 %) as indicated on the bottle and vertex thoroughly. Buffer CW1, Buffer CW2 and Buffer CLB-3 which are stable for at least 18 months after the addition of ethanol, when stored at room temperature (15-25 °C).
- Dissolve Proteinase K as indicated on the bottle with the help of Protease Dissolve Buffer, store at -20 °C after dissolve.
- Dissolve Carrier RNA as indicated on the bottle with the help of Buffer AE, store at -20 °C after dissolve.

Protocol

A. Circulating Cell free DNA Purification from Plasma/Serum

1). Pipette 100 µl of Proteinase K into a 5 ml microcentrifuge tube.

2). Add 1 ml of serum or plasma to the tube, mix for 5 sec.

3). Add 0.8 ml of Binding Buffer and 5 µl of Carrier RNA to the tube, close the cap and mix thoroughly by pulse-vortexing for 15 sec. Incubate at 60 °C for 30 min.

4). Add 1.8 ml of Buffer CLB-3 to the lysate in the tube, close the cap and mix thoroughly by pulse-vortexing for 30 sec. Incubate the lysate-Binding Buffer mixture in the tube for 5 min on ice.

5). Insert a Mini Column in a 2 ml Collection Tube.

6). Add up to 750 µl of the solution from Step 4 to the Column. Centrifuge at 8,000 x*g* for 1 min at room temperature. Discard the filtrate and reuse the collection tube.

7). Repeat Step 6 until all of the sample has been transferred to the column. Discard the filtrate and the collection tube.

8). Insert the column in a new 2 ml Collection Tube. Add 650 µl of Buffer CW1 to the column. Centrifuge at 12,000 x*g* for 1 min. Discard the filtrate and reuse the collection tube.

9). Add 650 µl of Buffer CW2 to the column. Centrifuge at 12,000 x*g* for 1 min. Discard the filtrate and reuse the collection tube.

10). Add 650 µl of absolute ethanol to the column. Centrifuge at 12,000 x*g* for 1 min at room temperature. Discard the filtrate and reuse the collection tube.

11). Centrifuge the empty column at 12,000 x*g* for 1 min at room temperature to dry the column matrix.

12). Place the Mini column in a clean 1.5 ml microcentrifuge tube. Open the lid and incubate the assembly at 56 °C for 10 min, to dry the membrane completely.

13). Add 30-50 µl of Buffer AE directly to the center of the column membrane, leave it undisturbed at room temperature for 3 min, and centrifuge at 12,000 x*g* for 1 min at room temperature.

14). Store the isolated DNA at -20 ºC for long term storage.

Troubleshooting guide

A. Poor or low yield of DNA

- **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 them.

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.

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
	IN VITRO DIAGNOSTIC MEDICAL DEVICE
	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
	AUTHORIZED REPRESENTATIVE IN THE EUROPEAN COMMUNITY/ EUROPEAN UNION
	EUROPEAN CONFORMITY

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EC REP

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