RUO

MAGNXT

Sepsis Direct Whole Blood Extraction Kit

S. No.	Product Name	SKU Code	Pack Size
1.	MagNXT Sepsis Direct Whole Blood Extraction Kit	G2MBR4-0888	50T
2.	MagNXT Sepsis Direct Whole Blood Extraction Kit	G2MBR4-0889	250T

Content Table

Intended Use

MagNXT Sepsis Direct Whole Blood Extraction Kit is an *in-vitro* screening test kit, intended for isolation of DNA from research samples such as Whole Blood for early and accurate detection of sepsis-causing pathogens by delivering high-quality DNA from whole blood quickly and efficiently. It is ideal for integration into molecular diagnostic workflows such as real-time PCR.

Intended User

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

Test Principle

MagNXT Sepsis Direct Whole Blood Extraction Kit utilizes a magnetic bead-based method for the rapid isolation of microbial nucleic acids directly from whole blood. The process begins with chemical lysis and enzymatic digestion to release DNA from cells. Magnetic beads are then added under specific binding conditions, allowing nucleic acids to adsorb to the bead surface. An external magnet is used to separate the beads from cellular debris and inhibitors. After a series of wash steps to remove impurities, nucleic acids are eluted in a low-salt buffer for use in downstream molecular applications such as PCR or isothermal amplification.

Summary

The Sepsis Direct Whole Blood Extraction Kit uses magnetic beads to isolate microbial DNA from whole blood. After lysis and digestion, nucleic acids bind to the beads, which are then magnetically separated, washed, and eluted. The purified nucleic acids are ready for downstream molecular testing like PCR or isothermal amplification.

Materials Required But Not Provided

- Micropipettes (Adjustable)
- Disposable barrier (Filter) pipette tips
- 1.5 ml microcentrifuge tubes
- Table top microcentrifuge
- Magnetic Separation Rack (can be supplied by Genes 2Me on request)
- Molecular biology grade ethanol (96-100%)
- Personal protective equipment (lab coat, gloves, goggles, etc)
- Disposable gloves
- Vortexer

Storage, Operating Conditions and Stability

- This product can be stored at room temperature (15°C - 25°C) for the duration of its shelf life, as indicated on the box label. 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. Magnetic Bead Particles shipped at room temperature and stored at 2°C - 8°C.
- After reconstitution, Proteinase K (20mg/ml) needs to be stored at -20°C.

Important Note:

- **Sample collection and storage:** Best results are obtained with fresh samples that has been immediately stored at appropriate temperature. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced quality of purified nucleic acid. Use of poor quality samples will lead to low yield of purified nucleic acid.

Instructions Before Use

- Buffer DW-Wash 1, Buffer DW-Wash 2 are supplied as concentrates. Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle and shake thoroughly.
- After addition of ethanol, Buffer DW-Wash 1, Buffer DW-Wash 2 can be stored at room temperature (15–25°C) for the duration of its shelf life, as indicated on the box label.
- Add Protease Dissolve Buffer into absolute amount of Proteinase K and store it at -20°C.

Sample Collection

- Collect patient whole blood sample in collection tubes according to the recommended instructions on the kit.
- Process samples within 72 hours, ideally within 36 hours for accurate Minimum Residual Disease measurement.
- Handle and store the samples carefully to maintain stability.
- Ensure consistent temperature during shipping to avoid changes.

Sample Preparation Protocol - Pre-Processing

Table 1. Pre-process Components

Kit Contents	Kit Content Code	Kit Content Quantity	Kit Content Code	Kit Content Quantity
	50 Tests	50 Tests	250 Tests	250 Tests
Cell crac Fluid	G2MBR3-1996-1	1 X 50 ml	G2MBR3-1996-2	1 X 250 ml
Catalyst Enzyme	G2MBR3-1997-1	1 X 1.5 ml	G2MBR3-1997-2	1 X 7.5 ml
Pathoenrich Buffer	G2MBR3-1998-1	1 X 1.5 ml	G2MBR3-1998-2	1 X 75 ml
Patho Sphere Bead Tube	G2MBR3-1999-1	50 X 1 T	G2MBR3-1999-2	250 X 1 T

Direct Whole Blood Protocol: Preprocessing

1). Transfer 1.0–1.5 ml of whole blood into a 2 ml sterile centrifuge tube.

2). Add 1 ml of CellCrac Fluid to the above step-01

3). Add 30 µl of Catalyst Enzyme to the tube & vortex for 15-30 seconds.

4). Incubate at RT (15–25°C) for 10 minutes to lyse host cells and release pathogen DNA.

5). Centrifuge at 10,000 x*g* for 10 minutes and carefully discard the supernatant.

6). Add 300 µl of Pathoenrich Buffer.

7). Transfer the entire mixture to a 2 ml PathoSphere Beads Tube and screw the lid tightly.

8). Vortex gently for 1–2 min for effective DNA enrichment.

9). Incubate at room temperature (15–25°C) for 5 min.

10).Centrifuge the tube at 13,000 x*g* for 3 min.

11).Use 200 µl of pre-processed sample & proceed with step-1 of manual extraction protocol.

MagNXT Sepsis Direct Whole Blood Extraction Kit

Platform-Manual

SKU Code- G2MBR4-0888-50T, G2MBR4-0889-250T

Table 2. Kit Components

Kit Contents	Kit Content Code	Kit Content Quantity	Kit Content Code	Kit Content Quantity
	50 Tests	50 Tests	250 Tests	250 Tests
Magnetic Bead Particles	G2MBR3-2000-1	1 X 1 ml	G2MBR3-2000-2	1 X 5 ml
Buffer DW-Lysis	G2MBR3-2001-1	1 X 23 ml	G2MBR3-2001-2	1 X 115 ml
Buffer DW-Wash 1	G2MBR3-2002-1	1 X 13 ml	G2MBR3-2002-2	1 X 60 ml
Buffer DW-Wash 2	G2MBR3-2003-1	1 X 10 ml	G2MBR3-2003-2	2 X 25 ml
Buffer AVE	G2MBR3-2004-1	1 X 3 ml	G2MBR3-2004-2	1 X 14 ml
Proteinase K (20mg/ml)	G2MBR3-2005-1	1 X 20 mg	G2MBR3-2005-2	1 X 100 mg
Protease Dissolve Buffer	G2MBR3-2006-1	1 X 2 ml	G2MBR3-2006-2	1 X 6 ml

Manual Protocol

1). Add 20 µl Proteinase K , 20 µl Magnetic Bead Particles and 450 µl Buffer DW-Lysis and vortex for 15 sec. Leave at room temperature for 10 min with several invert mix. Transfer to a magnetic stand, and leave it undisturbed for ~3 min to adsorb the magnetic beads. carefully discard the supernatant.

2). Add 500 µl Buffer DW-Wash 1 and vortex for 10 sec. Transfer the microcentrifuge tubes into a magnetic stand and leave it undisturbed for ~1 min to adsorb magnetic beads. Carefully discard the supernatant.

3). Add 500 µl Buffer DW-Wash 2 and vortex for 10 sec. Transfer the microcentrifuge tubes to a magnetic stand and leave it undisturbed for 1 min to adsorb magnetic beads. Completely remove and discard the cleared supernatant.

4). Repeat step 4 again.

5). Discard all the leftover supernantant, and air dry the pellet for ~10 min.

6). Add 50 µl Buffer AVE to the pellet and vortex for proper mixing. Leave it undisturbed for 5 min, vortex several times for efficient mixing.

7). Transfer to a magnetic stand and leave it undisturbed for 3 min. Transfer the extracted elute (DNA) to a new 1.5 ml microcentrifuge tube & stored at -20°C.

Troubleshooting Guide

A. Poor yield / low sensitivity

1) Incomplete sample lysis

- Sample mixed with Lysis Buffer and was not thoroughly homogenized. The mixture has to be shaken continuously. Alternatively, prolong the incubation time with Lysis Buffer.

2) Insufficient elution buffer volume

- Bead pellet must be covered completely with elution buffer and needs to be fully resuspended. Insufficient performance of elution buffer during elution step.
- Remove all buffers completely from the bead pellet after the binding and wash steps. Remaining buffer decreases the efficiency of the subsequent steps. Aspiration of adsorbed bead pellet.
- Do not disturb the attracted beads while aspirating the supernatant. This requires special caution when removing the lysate from the beads as the lysate is usually too opaque to allow visual control of the pellet.
- Time for magnetic separation too short or aspiration speed too high.

B. Low Purity/Low Sensitivity Insufficient washing procedure

- Make sure that beads are resuspended completely during the washing procedure. If shaking is not sufficient to resuspend the beads completely mix by repeated pipetting up and down.

C. Poor performance of Nucleic acid in down-stream applications Carry-over of ethanol from wash buffers

- Be sure to remove all of the 80% ethanolic wash solution from the final wash, as residual ethanol interferes with downstream applications. Ethanol evaporation from wash buffers.
- Close the buffer bottles tightly, avoid ethanol evaporation from buffer bottles as well as from buffer filled in reservoirs. Do not reuse buffer reservoirs.

Limitations

- **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.
- **Processing Time:** The extraction process can be time-consuming, particularly when dealing with multiple types of body fluids. This can lead to workflow bottlenecks, especially in high-throughput settings. Efficient workflow management and optimization of extraction protocols are necessary to minimize processing time and increase productivity.
- **Operator Skill:** The efficiency of the extraction process may vary depending on the expertise of the operator. Variability in results between different users can occur if operators lack sufficient training or experience. Standardized protocols and regular training sessions can help ensure consistency in results across different operators.

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
RUO	RESEARCH USE ONLY
	UPWARD
	CONSULT INSTRUCTIONS FOR USE
LOT	BATCH CODE
REF	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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