TIANLONG

Nucleic Acid Extraction Kit

(For Bacteria Genomic DNA Extraction)

User Guide

Σ 20 T131H

Σ 64 T132H

Σ 20 T134H

Version 7.0

CE IVD

In-Vitro Diagnostics / For use with Automatic nucleic acid extractor compatible with Bacteria Genomic DNA Extraction Kit

REF T131H T132H T134H

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Contents

Kit Version	7.0		
Changes	Address of Manufacturer Address of EU Representative Chapter “Intended Use” Chapter “Kit Contents” Chapter “Warnings and Precautions” Chapter “1. Automated Extraction Process” Chapter “Limitations of Test Methods” Chapter “Safety Symbols and Signs” Small lexical corrections.	Additions	/

Intended Use

The ***Bacteria Genomic DNA Extraction Kit*** is intended for rapidly extracting genomic DNA from bacterial suspension cultures, cotton swabs, sputum, body fluids and stool samples. The extracted genomic DNA is of high purity and stability and can be used in a variety of routine operations, including enzyme digestion, Polymerase Chain Reaction (PCR), DNA library constructions, Southern hybridization and blotting and other experiments.

The ***Bacteria Genomic DNA Extraction Kit*** is intended to be used by professionals, such as biotechnologists, microbiologists, clinical technicians, and physicians who are trained in molecular and biological techniques.

Product Performance Indicators

The extraction kit can extract total yield of DNA from 1 mL sample ≥ 2 μg.
Both the Extraction Purity: OD260/OD280 ≥ 1.5.

Special Notes

The ***Bacteria Genomic DNA Extraction Kit*** is worked with TIANLONG® automatic nucleic acid extractors (Libex , GeneRotex 96 and PANA 9600S)that have been disinfected by UV light before use. After an experiment, wipe the inside of the extractor with 75% ethanol and disinfect it with UV light for 15 mins. An automatic nucleic acid extractor automates the entire purification process and can process 1-96 samples in a single run.

The ***Bacteria Genomic DNA Extraction Kit*** is used to extract genomic DNA from bacterial suspension cultures, cotton swabs, sputum, body fluids and stool samples. Use exclusive-use utensils and sample injectors and use disposable centrifuge tubes and tips processed by autoclave before using. The operator should wear powder-free gloves and a mask and a protective cover all.

The kit has magnetic beads with a unique separation function and a unique buffer system to extract, isolate and purify high-quality nucleic acids from bacterial suspension cultures, cotton swabs, sputum, body fluids and stool samples.

Magnetic beads enable the purification of high-quality nucleic acids that are free of protein, nuclease, and other impurities. Purified nucleic acids can be widely used in a variety of routine operations, including downstream experiments such as enzyme digestion, Polymerase Chain Reaction (PCR), DNA library construction, and Southern hybridization and blotting.

Please carefully read the manual of instructions before attempting to install or use the product for the first time. To consider all possible consequences of incorrect operation or non-recommended functions, pay special attention to the possible consequences.

Testing Principle

The ***Bacteria Genomic DNA Extraction Kit*** is worked with TIANLONG® automatic nucleic acid extractors (Libex, GeneRotex 96 and PANA 9600S). During the nucleic acid extraction process. Magnetic beads are adsorbed, transferred and released using special magnetic rods based on the principle of magnetic bead adsorption. This enables the transfer of magnetic beads/nucleic acids, the automatic completion of the nucleic acid extraction, and final isolation of high-purity nucleic acids.

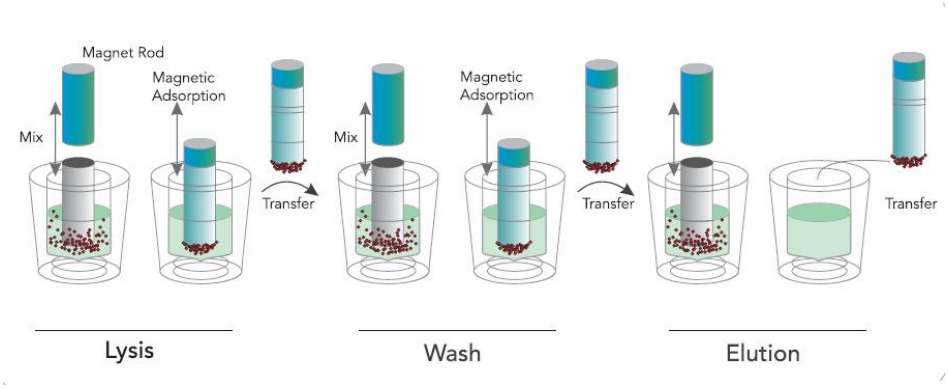

Figure 1. Schematic Diagram of Automatic Nucleic Acid Extractor

An automatic nucleic acid extractor performs the following steps on a sample containing magnetic particles:

A magnetic rod protected by the mixing sleeve inserts into a well which contains samples. The mixing sleeve stirs rapidly and repeatedly in the liquid to ensure complete mixing of the liquid and magnetic beads. After cell lysis, nucleic acid adsorption, washing and elution, highly pure nucleic acid is obtained.

Content of the Kit

Short Code / Name of Component		T131H	T132H	T134H
REAG1	Size	20 T/Box (Pre-filled)	64 T/Box (Pre-filled)	20 T/Box (Pre-filled)
	Component	Pre-filled 96-deep well plate	Pre-filled 96-deep well plate	Pre-filled 6 strip tube
	Quantity	4	4	20
	Component Specification	5 Tests	16 Tests	1 Test
REAG2	Component	Proteinase K Solution	Proteinase K Solution	Proteinase K Solution
	Component Specification	0.4 mL	1.28 mL	0.4 mL
	Quantity	1	1	1
REAG3	Component	Lysozyme	Lysozyme	Lysozyme
	Component Specification	64 mg (Dry powder)	64 mg (Dry powder)	64 mg (Dry powder)
	Quantity	2	4	2
REAG4	Component	Lysozyme Diluent	Lysozyme Diluent	Lysozyme Diluent
	Component Specification	6.4 mL	12.8 mL	6.4 mL
	Quantity	1	1	1
REAG5	Component	Bacteria Digestive Buffer	Bacteria Digestive Buffer	Bacteria Digestive Buffer

	Component Specification	4mL	12.8mL	4mL
	Quantity	1	1	1
Instructions for Use		1 Copy	1 Copy	1 Copy

Materials Required but not Provided

When working in a laboratory, make sure to wear a proper lab coat, powder-free disposable gloves and protective goggles. For more information, please consult the Safety Data Sheet (SDS) available from the product supplier.

- Pipettor: 20 µL, 200 µL or 1000 µL
- Tip: 20 µL, 200 µL or 1000 µL
- Vortex mixer
- High-speed centrifuge
- Metal bath
- Pipet
- PBS or normal saline, 1M sodium hydroxide
- Sample holder
- 75% ethanol
- Single kit docking (matched with T134H (6 strip tube), could be purchased from Tianlong.)
- Extractor

Warnings and Precautions

Please be sure to read the precautions before using the kit.

The extraction kit is particularly used for genomic DNA from bacterial suspension cultures, cotton swabs, sputum, body fluids and stool samples. Use exclusive-use utensils and sample injectors and use disposable centrifuge tubes and tips processed by autoclave before using. The operator (researcher or clinical expert) should wear powder-free gloves and a mask.

Please read the manual carefully before using the kit, and strictly follow the manual throughout operations. The clinical samples should be collected on a clean bench or in a bio-safety cabin.

Before using TIANLONG® automatic nucleic acid extractors (Libex, GeneRotex 96 and PANA 9600S), they must be disinfected by UV light. After an experiment, wipe the inside of the extractor with 75% ethanol and disinfect it with UV light for 15 mins.

Due to the possibility of residual magnetic beads in the eluate following extraction, every possible effort should be made to avoid suctioning of any magnetic beads during eluate absorption.

Do not mix reagents from different batches and use the kit within expiry date.

Dispose of all samples and reagent materials used in an experiment, and thoroughly clean and disinfect the experimental workbench.

The ***Bacteria Genomic DNA Extraction Kit*** is intended for in vitro diagnosis use.

When using the kit, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These documents are available online in a convenient and compact PDF format at https://www.medtl.net/resources/download/catalogue-all/catalogue, where the operator can find, view and print the appropriate MSDSs.

⚠ **Caution: Do not add any bleach or acidic solution directly to the pre-filled reagent.**

The pre-filled reagent contains guanidinium salts, which, when combined with bleach can form highly reactive compounds. If any of these buffers are spilled, clean immediately with a suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with sodium hypochlorite at a concentration of 1% (v/v). The kit comes with the following warnings and precautions.

Name of Component		Hazard pictograms (CLP)	Classification under CLP:	H- and P-statements
REAG 1	Lysis Buffer Washing Buffer A		Acute toxicity (oral), Category 4 Skin corrosion/irritation, Category 2 Serious eye damage/eye irritation, Category 2	**Hazard statements (CLP)** H302: Harmful if swallowed. H315:Causes skin irritation. H319:Causes serious eye irritation. **Precautionary statements (CLP)** P264 **:** Wash hands, forearms and face thoroughly after handling. P280:Wear protective gloves/protective clothing/eye protection/face protection/hearing protection. P321:Specific treatment (see supplemental first aid instruction on this label). P337+P313:If eye irritation persists: Get medical advice/attention. P501:Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.
	Magnetic Beads Dilution Buffer Washing Buffer B Washing Buffer C Elution Buffer	None	None	None
REAG 2	Proteinase K Solution	None	None	None
REAG 3	Lysozyme		Respiratory sensitisation, Category 1	**Hazard statements (CLP)** H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled. **Precautionary statements (CLP)** P261 - Avoid breathing dust/fume/gas/mist/vapours/spray. P284 - Wear respiratory protection. P304+P340 - IF INHALED: Remove person to fresh air and keep comfortable for breathing. P342+P311 - If experiencing respiratory symptoms: Call a POISON CENTER or doctor. P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.
REAG 4	Lysozyme Diluent	None	None	None

REAG 5	Bacteria Digestive Buffer	None	None	None

Please see MSDS for more details.

Precautions for Safe Handling

Do not dispose of the preparations or of the packaging waste in drains leading to the sewage system or in the drainage system for waste not produced by industrial processing/analysis waste.

Any material in contact with reagents should be treated as a biological contaminant and treated in accordance with relevant local regulations.

Reagent Storage and Handling

The kit should be stored at room temperature in a cool, dry and well-ventilated area. All components of the kit can be adequately stored for up to 12 months.

The kit should be used in a well-ventilated area, keep away from the source of heat, sparks, open flames, and smoking.

To avoid evaporation, the pre-filled reagent should be used immediately after open, and should not be placed for a long period of time.

Avoid exposure to UV light (e.g., for decontamination), which may result in accelerated aging.

Sample Handling and Storage

Prevent foam formation inside or on the samples. Depending on the starting material, sample pre-treatment may be required. Samples should be stored at room temperature (15~25°C) before starting the experiment.

Samples should be used immediately after collection to extract nucleic acid or stored at 2~8°C for further experiment within 24 hours. While for long-term storage, the samples should be placed at -20°C.

For detailed information on sample pretreatment, please refer to 2.1.3.

Operation Guide

1. Automated Extraction Process

Automatic nucleic acid extractors (Libex, GeneRotex 96 and PANA 9600S) enable nucleic acid extraction by magnetic beads. They use magnetic rods to move the beads adsorbed with nucleic acid into different reagent wells.Magnetic rod protected by the mixing sleeve which stirs rapidly and repeatedly in the liquid to ensure complete mixing of the liquid and magnetic beads. After cell lysis, nucleic acid adsorption, washing, and elution, the highly pure nucleic acids are obtained. Automatic nucleic acid extractors are characterized by high automation, rapid extraction speed, stable results, and ease of operation.
The user needs to load samples and magnetic bead nucleic acid extraction reagents into the reaction consumables, the nucleic acid extractors are going to perform all nucleic acid extraction operations according to the experimental procedures. Please refer to the user manual provided with an instrument for operating instructions.

2. Operation Steps of Automated Extraction

2.1 Automatic Nucleic Acid Extractor (model: Libex)

2.1.1 Edit Experiment Program

The extraction procedure of Libex Nucleic Automatic Acid Extractors is as follows:

No.	Well	Name	Waiting (s)	Mixing (s)	Magnet (s)	Speed	Volume (μL)	Heating State	Temp (°C)
1	1	Lysis	0	1200	0	5	600	Lysis	90
2	2	Remove Bead	0	60	60	7	600	Closed	0
3	1	Combine	0	600	90	7	600	Lysis	90
4	3	Washing1	0	120	90	7	600	Closed	0

5	4	Washing2	0	120	90	7	600	Elution	80
6	5	Washing3	0	0	30	7	600	Elution	80
7	6	Elution	0	300	90	7	100	Elution	80
8	2	Release Bead	0	60	0	7	600	Closed	0

2.1.2 Reagent Preparation

96-deep well plate: Open the kit and take out the REAG1, slowly invert it several times to resuspend the magnetic beads, then remove the plastic package and gently shake the 96-well plate so that the reagent and magnetic beads are concentrated on the bottom of the 96-well plate (a 96-well plate horizontal centrifuge can also be used for centrifugation at 500 rpm for 1 min). Carefully tear off the aluminum foil sealing film before use to avoid plate vibration and liquid splashing.

6 strip tube: Open the kit and take out the REAG1, slowly invert it several times to resuspend the magnetic beads, then gently shake the 6 strip tube so that the reagent and magnetic beads are concentrated on the bottom of the tube. Put the reagent on the docking (note the direction and make sure that the tube is placed at the lowest level), carefully tear off the aluminum foil sealing film before use to avoid plate vibration and liquid splashing, which is shown in Figure 2.

Figure 2. Put the 6 strip tube on the singe kit docking

2.1.3 Adding Sample to the Reagent

Lysozyme solution preparation: Add 3.2 mL REAG4 to each REAG3 and mix thoroughly. Dissolved Lysozyme should be stored at -20°C or not more than 6 hours at room temperature, avoid repeated freezing and thawing (no more than 5 times).

Sample beneficiation

Bacterial suspension cultures: Pipet 1-3 mL bacterial culture into a microcentrifuge tube and centrifuge for 1 min at 10000 rpm, and remove supernatant by pipetting.

Bacterial cotton swabs: Put the collected cotton swabs in appropriate PBS (or normal saline) and mix by vortexing. Incubate for 5-10 mins at room temperature. Pipet supernatant into a microcentrifuge tube and centrifuge for 10 mins at 7500 rpm and remove supernatant by pipetting.

Bacterial sputum: Pipet 0.5-1 mL bacterial sputum into a microcentrifuge tube and add 1 mL 1M sodium hydroxide. Mix thoroughly by vortexing and centrifuge for 10 mins at 12000 rpm, remove supernatant by pipetting. Put 1 mL PBS to the centrifuge tube and after gently mixing and centrifuge for 10 mins at 12000 rpm, then remove supernatant.

Bacterial body fluids (such as urine): Pipet bacterial body fluids into a microcentrifuge tube and centrifuge for 10 mins at 7500 rpm, then remove supernatant by pipetting.

Bacterial stool: Add 1 mL PBS (or normal saline) to a microcentrifuge tube that contains 0.2g stool. Mix continuously by vortexing and centrifuge for 5 mins at 500 rpm and collect the supernatant. Repeat this above step twice. Collect all the supernatant for 10 mins at 5000 rpm and remove supernatant by pipetting.

Sample pretreatment (If the REAG5 is precipitated, please incubate at 37°C for dissolution and shake thoroughly.)

Gram-negative bacteria genomic DNA: Add 200 µL REAG5 and 20µL REAG2 to the microcentrifuge tube containing the preparative product of the above bacterial samples. Mix thoroughly by vortexing and pipet

all digestion mixture into the 1st and 7th column of the 96-deep well plate (note the column no. is for effective wells), or the 1st column of the 6 strip tube.

Gram-positive bacteria genomic DNA: Add 180 µL Lysozyme solution and 20 µL REAG2 to the microcentrifuge tube that contains the preparative product of above bacterial samples. Mix thoroughly by vortexing and incubate for at least 30 mins at 50°C (incubation time depends on bacterial species). Briefly centrifuge and add 200 µL REAG5 to the microcentrifuge tube. Mix again by vortexing and pipet all digestion mixture into 1st and 7th column of the 96-deep well plate (note the column no. is for effective wells), or 1st column of the 6 strip tube.

Extraction steps of Un-known bacteria genomic DNA: We suggest following extraction steps of gram-positive bacteria genomic DNA.

⚠ **Caution: When pipetting the sample, avoid having substance than liquid adhere to the tip of the sample injector; do not add the sample too quickly to avoid contaminating the upper portion of the well wall; and do not splash air bubbles to avoid contaminating adjacent wells.**

Note: The following points should be taken into consideration when determining whether a sample is suitable for the Bacteria Genomic DNA Extraction Kit.

a. Type of sample: As stated in the intended use.

b. Short-term storage: Samples can be used immediately after collection for nucleic acid extraction or stored at 2~8°C for testing with a maximum storage period of 24 hours.

c. Long-term storage: If the user does not operate the sample temporarily, it should be kept sealed in a refrigerator at -20°C.

2.1.4 Loading in Deep Well Plate

Place the 96-deep well plate or 6 strip tube in the Automatic Nucleic Acid Extractor and ensure the marked notch of the plate faces front.

Insert the mixing sleeve into the sleeve holder and close the cabin door.

Note: As shown in Figure 3 and Figure 4, the user should ensure that the 96-deep well plate and the single kit docking is properly positioned with the notch facing outward.

Note: Insert the 96-deep well plate or the single kit docking into the experiment cabin and push the magnetic rod covers into the right position. Check the position of the magnetic rod covers. Otherwise, instrument dysfunction or malfunction may occur and affect the experiment results.

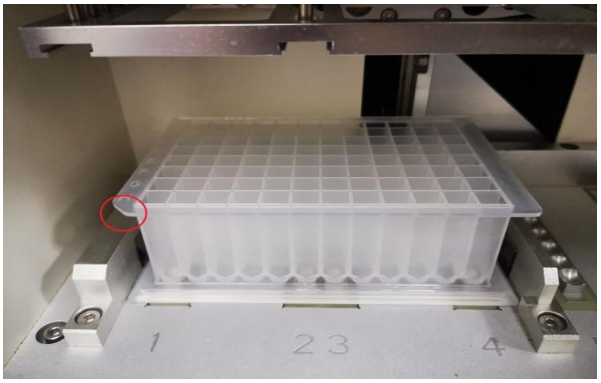

Figure 3. 96-deep well plate

Figure 4. Put the single kit docking into the instrument

2.1.5 Procedure Run

Program operation see 2.1.1 for specific programs. After the procedure is completed, the instrument will notice the user the experiment has completed. Transfer the extracted product from column 6 and column 12 to a clean centrifuge tube which is free of nuclease.

Note: If the user does not analyze the extracted product for the immediate use, please store it sealed in a refrigerator at -20°C.

⚠ **Caution: Any used deep well plate and mixing sleeves should be considered as biological contaminants and disposed of in accordance with relevant regulations.**

⚠ **Caution: Using expired reagents or those that are not compatible with this instrument does not guarantee that the expected results will be obtained.**

2.1.6 Cleaning and Maintenance of the Instrument

Follow the Cleaning and Maintenance of the Instrument section in accordance with the instruction in the user manual provided with the equipment. Ensure that the experimental cabin is cleaned regularly to minimize the risk of cross-contamination.

2.2 Automatic Nucleic Acid Extractor (model: GeneRotex 96)

2.2.1 Edit Experiment Program

The extraction procedure of GeneRotex 96 Nucleic Automatic Acid Extractor is as follows:

Step	Name	Well	Stir (min:s)	Magnetic (min:s)	Wait (min:s)	Speed (rpm)	Volume (μL)	T Control (°C)
1	Lysis	1	20:00	00:00	00:00	2500	500	110
2	Remove Bead	2	01:00	01:00	00:00	2500	600	0
3	Combine	1	10:00	01:30	00:00	2500	500	110
4	Washing 1	3	03:00	01:30	00:00	2500	700	0
5	Washing 2	4	02:00	01:30	00:00	2500	700	100
6	Washing 3	5	00:00	00:30	00:00	2500	700	100
7	Elution	6	05:00	01:30	00:00	2500	100	100

2.2.2 Reagent Preparation

96-deep well plate:

Open the kit and take out the REAG1, slowly invert it several times to resuspend the magnetic beads, then remove the plastic package and gently shake the 96-well plate so that the reagent and magnetic beads are concentrated on the bottom of the 96-well plate (a 96-well plate horizontal centrifuge can also be used for centrifugation at 500 rpm for 1 min). Carefully tear off the aluminum foil sealing film before use to avoid plate vibration and liquid splashing.

6 strip tube:

6 strip tube: Open the kit and take out the REAG1, slowly invert it several times to resuspend the magnetic beads, then gently shake the 6 strip tube so that the reagent and magnetic beads are concentrated on the bottom of the tube. Put the reagent on the docking (note the direction and make sure that the tube is placed at the lowest level), carefully tear off the aluminum foil sealing film before use to avoid plate vibration and liquid splashing, which is shown in Figure 2.

2.2.3 Adding Sample to the Reagent

Lysozyme solution preparation: Add 3.2 mL REAG4 to each REAG3 and mix thoroughly. Dissolved Lysozyme should be stored at -20°C or not more than 6 hours at room temperature, avoid repeated freezing and thawing (no more than 5 times).

Sample beneficiation

Bacterial suspension cultures: Pipet 1-3 mL bacterial culture into a microcentrifuge tube and centrifuge for 1 min at 10000 rpm, and remove supernatant by pipetting.

Bacterial cotton swabs: Put the collected cotton swabs in appropriate PBS (or normal saline) and mix by vortexing. Incubate for 5-10 mins at room temperature. Pipet supernatant into a microcentrifuge tube and centrifuge for 10 mins at 7500 rpm and remove supernatant by pipetting.

Bacterial sputum: Pipet 0.5-1 mL bacterial sputum into a microcentrifuge tube and add 1 mL 1M sodium hydroxide. Mix thoroughly by vortexing and centrifuge for 10 mins at 12000 rpm, remove supernatant by pipetting. Put 1 mL PBS to the centrifuge tube and after gently mixing and centrifuge for 10 mins at 12000 rpm, then remove supernatant.

Bacterial body fluids (such as urine): Pipet bacterial body fluids into a microcentrifuge tube and centrifuge for 10 mins at 7500 rpm, then remove supernatant by pipetting.

Bacterial stool: Add 1 mL PBS (or normal saline) to a microcentrifuge tube that contains 0.2g stool. Mix continuously by vortexing and centrifuge for 5 mins at 500 rpm and collect the supernatant. Repeat this above step twice. Collect all the supernatant for 10 mins at 5000 rpm and remove supernatant by pipetting.

Sample pretreatment (If the REAG5 is precipitated, please incubate at 37°C for dissolution and shake thoroughly.)

Gram-negative bacteria genomic DNA: Add 200 µL REAG5 and 20µL REAG2 to the microcentrifuge tube containing the preparative product of the above bacterial samples. Mix thoroughly by vortexing and pipet all digestion mixture into the 1st and 7th column of the 96-deep well plate (note the column no. is for effective wells), or the 1st column of the 6 strip tube.

Gram-positive bacteria genomic DNA: Add 180 µL Lysozyme solution and 20 µL REAG2 to the microcentrifuge tube that contains the preparative product of above bacterial samples. Mix thoroughly by vortexing and incubate for at least 30 mins at 50°C (incubation time depends on bacterial species). Briefly centrifuge and add 200 µL REAG5 to the microcentrifuge tube. Mix again by vortexing and pipet all digestion mixture into 1st and 7th column of the 96-deep well plate (note the column no. is for effective wells), or 1st column of the 6 strip tube.

Extraction steps of unknown bacteria genomic DNA: We suggest following extraction steps of gram-positive bacteria genomic DNA.

⚠**Caution: When pipetting the sample, avoid having substance than liquid adhere to the tip of the sample injector; do not add the sample too quickly to avoid contaminating the upper portion of the well wall; and do not splash air bubbles to avoid contaminating adjacent wells.**

Note: The following points should be taken into consideration when determining whether a sample is suitable for the Bacteria Genomic DNA Extraction Kit.

a. Type of sample: As stated in the intended use.

b. Short-term storage: Samples can be used immediately after collection for nucleic acid extraction or stored at 2~8°C for testing with a maximum storage period of 24 hours.

c. Long-term storage: If the user does not operate the sample temporarily, it should be kept sealed in a refrigerator at -20°C.

2.2.4 Loading in the Deep Well Plate

Properly position the 96-deep well plate or 6 strip tube containing the sample in the experimental cabin of the automatic nucleic acid extractor (GeneRotex 96).

Note: The user should ensure that the 96-deep well plate should be placed with its notch at the upper left corner, as shown in Figure 5 and Figure 6.

Insert the rotatory mixing sleeves into column 2 and/or column 8 of the deep well plate and close the experimental cabin door.

⚠**Caution: The user must ensure that the rotatory mixing sleeves are placed properly; otherwise, the instrument may operate abnormally, or the magnetic rods may become contaminated.**

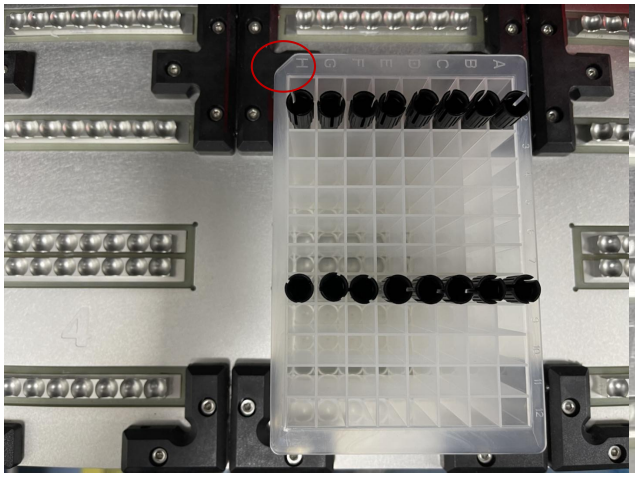

Figure 5. 96-deep well plate

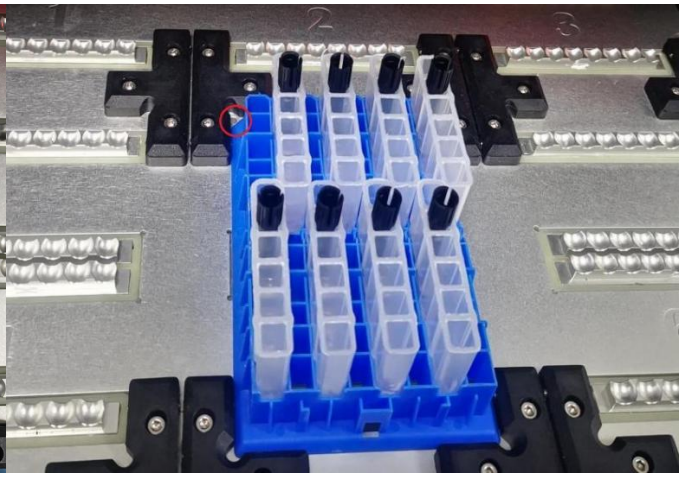

Figure 6. Put the single kit docking into the instrument

2.2.5 Experimental Procedure Run

Program operations see 2.2.1 for specific programs. After an experiment is run, the instrument will notify the user that the experiment is completed. Transfer the eluate from columns 6 and 12 to a clean centrifuge tube free of nuclease.

Note: If the user does not analyse the extracted product for the moment, please store it sealed in a refrigerator at -20°C.

2.2.6 Cleaning and Maintenance of the Instrument

Follow the Cleaning and Maintenance of Instrument in accordance with the user manual provided with the equipment. Ensure that the experimental cabin is cleaned regularly to minimize the risk of cross-contamination.

2.3 Automatic Nucleic Acid Workstation (model: PANA 9600S)

2.3.1 Experiment Preparation

Reagent Preparation

Please remove the PCR reagent from the refrigerator, thaw and balance to room temperature.

Lysozyme solution preparation: Add 3.2 mL Lysozyme diluent to each Lysozyme tube and mix thoroughly. Dissolved Lysozyme should be stored at -20°C or not more than 6 hours at room temperature, and avoid repeated freezing and thawing (no more than 5 times).

Adding Sample to the Reagent

- Please firstly record the sample information according to the requirements of laboratory operation.
- Please complete the sample centrifugation and other pre-processing operations according to the experimental requirements, and add or divide the prepared samples into sample tubes in the biosafety cabinet.
- Please insert the sample tubes into the sample holder and slowly push the sample holder along the track into the sample cabin.

Bacterial suspension cultures: Pipet 1-3 mL REAG4 to each REAG3 tube and centrifuge for 1 min at 10000 rpm, and remove supernatant by pipetting.

Bacterial cotton swabs: Put the collected cotton swabs in appropriate PBS (or normal saline) and mix by vortexing. Incubate for 5-10 mins at room temperature. Pipet supernatant into a microcentrifuge tube and centrifuge for 10 mins at 7500 rpm and remove supernatant by pipetting.

Bacterial sputum: Pipet 0.5-1 mL bacterial sputum into a microcentrifuge tube and add 1 mL 1M sodium hydroxide. Mix thoroughly by vortexing and centrifuge for 10 mins at 12000 rpm, remove supernatant by pipetting. Put 1 mL PBS to the centrifuge tube and after gently mixing and centrifuge for 10 mins at 12000 rpm, then remove supernatant.

Bacterial body fluids (such as urine): Pipet bacterial body fluids into a microcentrifuge tube and centrifuge for 10 mins at 7500 rpm, then remove supernatant by pipetting.

Bacterial stool: Add 1 mL PBS (or normal saline) to a microcentrifuge tube that contains 0.2g stool. Mix continuously by vortexing and centrifuge for 5 mins at 500 rpm and collect the supernatant. Repeat this above step twice. Collect all the supernatant for 10 mins at 5000 rpm and remove supernatant by pipetting.

Sample pretreatment (If the REAG5 is precipitated, please incubate at 37°C for dissolution and shake thoroughly.)

Gram-negative bacteria genomic DNA: Add 200 µL REAG5 and 20µL REAG2 to the microcentrifuge tube containing the preparative product of the above bacterial samples. Mix thoroughly by vortexing and pipet all digestion mixture into the 1st and 7th column of the 96-deep well plate (note the column no. is for effective wells), or 1st column of the 6 strip tube.

Gram-positive bacteria genomic DNA: Add 180 µL Lysozyme solution and 20 µL REAG2 to the microcentrifuge tube that contains the preparative product of above bacterial samples. Mix thoroughly by vortexing and incubate for at least 30 mins at 50°C (incubation time depends on bacterial species). Briefly centrifuge and add 200 µL REAG5 to the microcentrifuge tube. Mix again by vortexing and pipet all digestion mixture into 1st and 7th column of the 96-deep well plate (note the column no. is for effective wells), or 1st column of the 6 strip tube.

Extraction steps of Un-known bacteria genomic DNA: We suggest following extraction steps of gram-positive bacteria genomic DNA.

Note: The following points should be taken into consideration when determining whether a sample is suitable for the Bacteria Genomic DNA Extraction Kit.

a. Type of sample: As stated in the intended use.

b. Short-term storage: Samples can be used immediately after collection for nucleic acid extraction or stored at 2~8°C for testing with a maximum storage period of 24 hours.

c. Long-term storage: If the user does not operate the sample temporarily, it should be kept sealed in a refrigerator at -20°C.

Consumable Preparation

- User can prepare the corresponding reagent and consumables and load them in the right position according to the requirement information of reagent and consumable.

2.3.2 Experiment Running

a. Pre-filled 96 deep well plate: Take out the REAG1, turn it up and down to suspend the magnetic beads. Then remove the vacuum package, gently swing the plates to make the magnetic beads are gathered at the bottom of the wells. Please carefully tear down the aluminum foil sealing membrane to avoid liquid splash.
b. Please follow the manual to set the protocol.

2.3.3 Experiment Complete

Product Transfer

- After the experiment, please add the PCR consumables and transfer the PCR reaction system established by the PANA workstation to the PCR equipment for follow-up experiment.
- After the experiment, please cover the sample reserve tubes and transfer the reserved sample of nucleic acid extracted from the PANA workstation to the -20°C refrigerator.

Reagent and Sample Recovery

- After the experiment, cover the reagent bottles and recover the remaining reagents from the reagent cabin of the PANA workstation and store them in -20°C refrigerator together with the code and the reagent holder.
- After the experiment, take out the sample holders, cover the sample tubes, and store the sample in the refrigerator.

Instrument Cleaning and Maintenance

After the experiment, consider the used consumables such as deep well plates, rod covers, premix bottles as biological contaminated and comply with all applicable local or national regulations for the disposal of potentially infected waste.

- After the experiment, please comply with all applicable local or national regulations, dispose the biological waste in the waste bin within the waste cabin of the PANA workstation, and replace the waste bag in the waste bin.

Troubleshooting Guide

This troubleshooting guide should assist you in resolving any problems that arise during the experimental process. For more information and Frequently Asked Questions, please visit our Technical Support Center at http://www.medtl.net. The scientists in our Tianlong company's Technical Services Department are always available to answer any questions you may have about the information and protocols contained in the manual, sample and assay technologies (Contact information is included on the back cover or at http://www.medtl.net).

When an exception or error occurs during the experiment, the current run step is terminated/stopped. After resolving the error or exception, restart the run from the beginning. The troubleshooting guide is shown in the following table.

No.	Fault Symptom	Fault Cause	Handling Method
1	The well plate vibrates and the liquid splashes when tearing off the aluminum foil sealing film.	When tearing the film, please click the well plate to prevent it from rocking.	The reagent for this plate shall be scrapped, and re-extraction shall be performed.
2	Add the sample to unexpected wells.	Please read this manual carefully before adding samples.	The reagent for this plate shall be scrapped, and re-extraction shall be performed.
3	The amount of liquid in the reagent wells is insufficient.	/	Contact the after-sales service of Tianlong.
4	Reuse of pre-filled components.	Please read the precautions in this manual before using the kit.	Perform re-extraction of nucleic acid.
5	Abnormal noise from the instrument during extraction	The 96-deep well plate may be placed incorrectly.	Conduct repositioning the deep well plate.
		The mixing sleeves may be inserted in wrong place.	Reinsert the stirring sleeve.
6	Poor extraction performance	Please follow the operation requirements in the manual	Contact the after-sales service of Tianlong.
		The temperature control components of the instrument may be abnormal.	Contact the after-sales service of Tianlong.
		Other	Contact the after-sales service of Tianlong.

* **Ensure that the reagents have been preserved and used according to the manufacturer's instructions.**

Quality Control

In accordance with Tianlong Company's ISO-certified Quality Management, each lot of The ***Bacteria Genomic DNA Extraction Kit*** is tested against predetermined specifications to ensure consistent product quality.

Limitations of Test Methods

The system performance has been established through performance evaluation studies using bacterial suspension cultures, cotton swabs, sputum, body fluids and stool to extract genomic DNA.

It is the user's responsibility to validate system performance for any procedures used in their laboratory that are not covered by the performance evaluation studies of Xi'an Tianlong Science and Technology Co., Ltd.

Although the kit is intended for use in public health and scientific research, the purity and quality of extraction results are also affected by the testing instruments and personnel.

The extraction kit is intended for use with clinical diagnostic samples, forensic materials, and scientific research samples. The instrument and operator have an effect on the concentration and purity of the extracted product. Any generated diagnostic results must be interpreted in conjunction with the other clinical or laboratory findings.

Safety Symbols and Signs

No.	Symbol	Implication
1		Catalogue number
2		Batch code
3		Contains sufficient for <N> tests
4		Use by date
5		Caution
6		Temperature limit
7		In vitro diagnostic medical device
8		Reminder
9		Manufacturer
10		Do not re-use
11		Conformed with EU standard
12		Authorized representative in the European Community
13	CONT	Content of the kit
14	REAG1	Pre-filled 96-deep well plate/6 strip tube
15	REAG2	Proteinase K Solution
16	REAG3	Lysozyme
17	REAG4	Lysozyme Diluent
18	REAG5	Bacteria Digestive Buffer
19		Warning
20		PAP21: Not-corrugated cardboard
21		Danger

Contact Information

For technical assistance and more information, please contact our Technical Support Center at +86-29-82682132 (Tel), +86-29-82216680 (Fax), inquiry@medtl.com or contact your local distributor.

For a patient/user/third party in the European Union and in countries with similar regulatory regime (Regulation 2017/746/EU on IVD Medical Devices); if, during the use of this device or as a result of its use, a serious incident has occurred, please report it to the manufacturer and/or its authorised representative and to your national regulatory authority.

For up-to-date licensing information or product-specific disclaimers, please see the respective User Guide. Tianlong User Guides are available at www.medtl.net or can be requested from Tianlong Technical Services or the local distributor.