TIANLONG

Nucleic Acid Extraction Kit
(For Plant Tissues Genomic DNA Extraction)
User Guide

Σ 20 T084H

Σ 64 T085H

Σ 20 T087H

Version 6.0

For use with automatic nucleic acid extractor compatible with Nucleic Acid Extraction Kit

REF T084H T085H T087H

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Intended Use

The ***Nucleic Acid Extraction Kit*** is intended for rapidly extracting genomic DNA from plant tissues samples (e.g. blade, pulp, seed, tuber etc.). 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 ***Nucleic Acid 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 efficiently extract nucleic acids from plant tissue sample.

The coefficient of variation (CV) of intra-assay and inter-assay for the extraction kit is less than 15%.

Special Notes

The ***Nucleic Acid Extraction Kit*** must be used in combination with TIANLONG® automatic nucleic acid extractors (Libex and GeneRotex 96) 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 1-32 samples in a single run.

The ***Nucleic Acid Extraction Kit*** is particularly used for targeted genomic DNA isolation. Therefore, all of experiment supplies, such as pipettes, tubes, tips, must be processed by autoclave. Operator should wear gloves and masks and protective coveralls.

The kit has magnetic beads with a unique separation function and buffer system to extract, separate and purify high-quality nucleic acids from plant tissues 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 the fields of diagnostics, genomics research, disease detection, food safety and forensic identification, etc.

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 ***Nucleic Acid Extraction Kit*** is worked with TIANLONG® automatic nucleic acid extractors (Libex and GeneRotex 96). During the nucleic acid extraction process, magnetic beads are adsorbed, transferred and released by special magnetic rods based on the principle of magnetic bead adsorption. The extraction process enables the conduction of nucleic acid extraction and final adsorption of highly pure nucleic acids with the transfer of magnetic beads and nucleic acids.

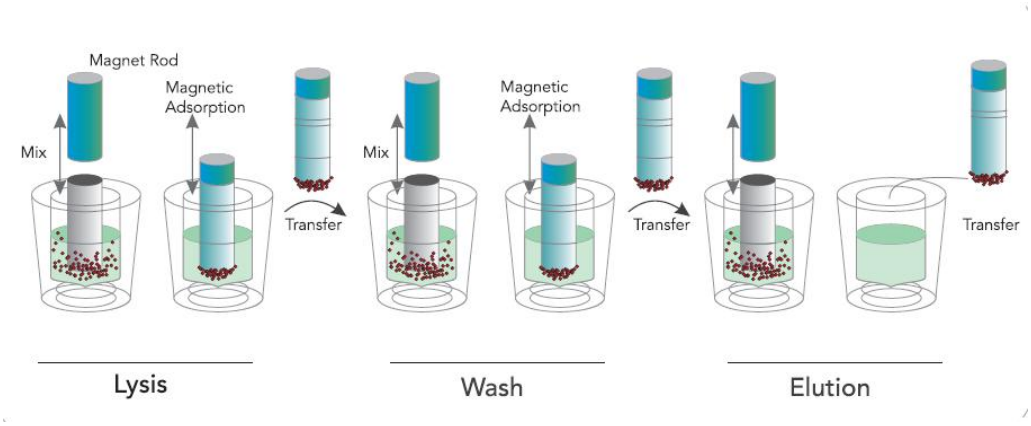

Figure 1. Schematic Diagram of Automatic Nucleic Acid Extractor

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

A magnetic rod protected by the mixing sleeve inserts into a well which contains sample. 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.

GeneRotex 96 is equipped with an array of 96 magnetic rods, allowing it to process up to 96 samples simultaneously.

Content of the Kit

Name of Component \ Short Code		T084H	T085H	T087H
REAG1	Size	20 T/Box	64 T/Box	20 T/Box
	Component	Pre-filled 96-deep well plate	Pre-filled 96-deep well plate	Pre-filled 6 strip tube
	Specification	5 Tests	16 Tests	1 Test
	Quantity	4	4	20
REAG2	Component	Pretreatment Reagent	Pretreatment Reagent	Pretreatment Reagent
	Specification	8 mL	25.6 mL	8 mL
	Quantity	1	1	1
REAG3	Component	Nucleic Acid Releaser	Nucleic Acid Releaser	Nucleic Acid Releaser
	Specification	1.2 mL	3.84 mL	1.2 mL
	Quantity	1	1	1
Corrugated Paper		1 Piece	1 Piece	1 Piece
White Board		1 Piece	1 Piece	1 Piece
Sponge Inner Support		1	1	1
Packaging Box		1	1	1
Instructions for Use		1 Copy	1 Copy	1 Copy
*Note: Ribonuclease A and Ribonuclease A Diluent should be purchased separately.				

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, 1000 µL
- Tip: 20 µL, 200 µL, 1000 µL
- Vortex mixer
- High-speed centrifuge
- Water bath or metal bath
- Sample holder
- 75% ethanol
- Single kit docking (matched with T087H (6 strip tube), can be purchased from Tianlong)
- Ribonuclease A and Ribonuclease A Diluent can 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 targeted genomic DNA isolation; therefore, all of experiment supplies, such as pipettes, tubes, tips, must be processed by autoclave. Operator should wear gloves and masks.

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

Before using TIANLONG® automatic nucleic acid extractors (Libex and GeneRotex 96), 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 clean and disinfect the experimental workbench thoroughly.

The ***Nucleic Acid Extraction Kit*** is intended for in vitro diagnosis use.

When using 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 first. Then clean 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 according to Regulation	Labelling according to Regulation
REAG 1	Lysis Buffer		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 A Washing Buffer B Washing Buffer C Elution Buffer	None	None	None
REAG 2	Pretreatment Reagent	None	None	None
REAG3	Nucleic Acid Releaser		Serious eye damage/eye irritation, Category 2	**Hazard statements (CLP)** H319 - Causes serious eye irritation. **Precautionary statements (CLP)** P280 - Wear protective gloves/protective clothing/eye protection/face protection/hearing protection. P337+P313 - If eye irritation persists: Get medical advice/attention.

Please see MSDS for more details.

Precautions for Safe Handling

Do not dispose of the preparations or 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 ***Nucleic Acid Extraction 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 opening 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

Avoid foam 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.

Detail information on sample pretreatment, please refer to 2.1.3.

1. Automated Extraction Process

Automatic nucleic acid extractors (Libex and GeneRotex 96) enable nucleic acid extraction by magnetic beads. They use magnetic rods to move the beads adsorbed with nucleic acid into different reagent wells and then rapidly and repeatedly stir the liquid through a mixing sleeve to mix the liquid and magnetic beads thoroughly. After cell lysis, nucleic acid adsorption, washing, and elution, the high-purity nucleic acid is obtained. Automatic nucleic acid extractors are characterized by high automation, rapid extraction speed, stable results, and ease of operation. They are compatible with special reaction consumables and can process up to 1-32 samples concurrently.

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.

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 Automatic Nucleic Acid Extractor is as follows:

No.	Well	Name	Waiting (s)	Mixing (s)	Magnet (s)	Speed	Volume (μL)	Heating State	Temp (°C)
1	2	Remove Bead	0	60	60	7	630	Closed	0
2	1	Lysis	0	900	90	7	700	Lysis	80
3	3	Washing 1	0	180	90	7	620	Closed	0
4	4	Washing 2	0	120	90	7	600	Elution	85
5	5	Washing 3	0	0	30	7	600	Elution	85
6	6	Elution	0	300	90	7	100	Elution	85
7	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. 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 liquid splashing.

6 strip tube:

Open the kit and take out the REAG1, slowly invert it several times to resuspend the magnetic beads. 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 splashing, which is shown in Figure 2.

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

2.1.3 Adding Sample to the Reagent

Ribonuclease A solution:

Dilute Ribonuclease A with Ribonuclease A dilution to final concentration of 20 mg/mL, it can be used after completely dissolved. The dissolved solution should storage at -20°C, and repeated freezing and thawing shall not exceed 5 times.

Sample Pretreatment

Take out appropriate amount of plant tissue from the fresh tissue 100 mg or dry weight 50 mg, different samples please refer to the table as below. Then placed it in the pre-cooled mortar, adding liquid nitrogen, fully crushed (In order to avoid thawing, please add liquid nitrogen constantly); after that transfer it to 1.5 mL sterile centrifugal tube.

Samples	Amount of samples
Fresh Plant Blade	50~100 mg
Dried Leaves	50~100 mg
Soybean Seed	100~200 mg
Wheat Seed	50~100 mg
Corn Seed	100~200 mg

Add 400 µL REAG2, and 60 µL REAG3, into 1.5 mL sterile centrifuge tube with plant tissue. Mix thoroughly 5-6 times upside down during the water bath (water bath: 65°C for 30mins). For dry weight tissue, water bath time should be appropriately extended; when the sample is seed, grind with a powder grind, then treat it with liquid nitrogen.

Remove the 1.5 mL sterile centrifuge tubes from the incubate, centrifuged 5 mins at 12000 rpm and the supernate is taken to be used for next step.

96 deep well plate: Add 300 µL supernate to column 1 and column 7 of the 96 deep well plate and add 20 µL Ribonuclease A solution to column 3 and column 9. (Be aware of the column No. is for effective wells).

6 strip tube: Add 300 µL supernate to column 1 of the pre-filled, and add 20 µL Ribonuclease A solution to column 3.

⚠ **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 *Nucleic Acid Extraction Kit.*

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

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 mixing sleeve holder and close the cabin door.

Note: As shown in Figure 3 and Figure 4, ensure that the 96-deep well plate and the single kit docking are properly positioned, and the marked notch of the plate faces front.

Note: Place the 96-deep well plate into the experiment cabin and push the mixing sleeves into the right position. Check the position of the mixing sleeves; otherwise, instrument dysfunction or malfunction may occur and affect the experiment results.

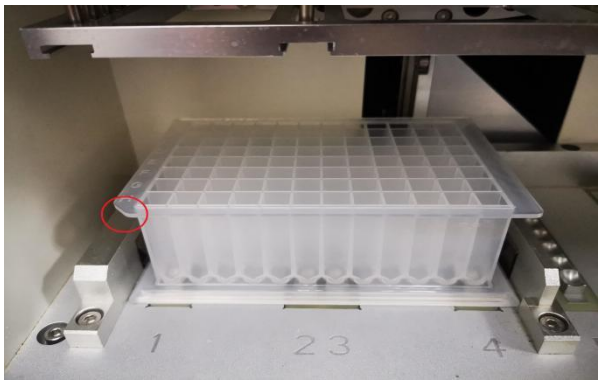

Figure 3. 96-deep well plate

Figure 4. Put the single kit docking into the instrument

2.1.5 Procedure Run

For special operations please see 2.1.1, After the procedure is completed, the instrument will notice the user the experiment has been completed. Transfer the extracted product from column 6 and column 12 to a clean centrifuge tube which is free of nuclease. (Be aware of the column No. is for effective wells)

Note: If the user does not analyse the extracted product immediately, please seal and store in a refrigerator at -20 °C.

Caution: Any used deep well plate and mixing sleeve 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 expected results.

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 Automatic Nucleic 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	Remove Bead	2	01:00	01:00	00:00	2500	630	0
2	Lysis	1	15:00	01:30	00:00	2500	700	90
3	Washing 1	3	03:00	01:30	00:00	2500	620	80
4	Washing 2	4	02:00	01:30	00:00	2500	600	80
5	Washing 3	5	00:00	00:30	00:00	2500	600	80
6	Elution	6	05:00	01:30	00:00	1500	100	80

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. 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 liquid splashing.

6 strip tube:

Open the kit and take out the REAG1, slowly invert it several times to resuspend the magnetic beads. 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 splashing, which is shown in Figure 5.

Figure 5. Put the 6 strip tube on the single kit docking

2.2.3 Adding Sample to the Reagent

Ribonuclease A solution:

Dilute Ribonuclease A with Ribonuclease A dilution to final concentration of 20 mg/mL, it can be used after completely dissolved. The dissolved solution should storage at -20 °C, and repeated freezing and thawing shall not exceed 5 times.

Sample Pretreatment

Take out appropriate amount of plant tissue from the fresh tissue 10 mg or dry weight 50 mg, different samples please refer to the table as below. Then placed it in the pre-cooled mortar, adding liquid nitrogen, fully crushed (In order to avoid thawing, please add liquid nitrogen constantly); after that transfer it to 1.5 mL sterile centrifugal tube.

Samples	Amount of samples
Fresh Plant Blade	50~100 mg
Dried Leaves	50~100 mg
Soybean Seed	100~200 mg
Wheat Seed	50~100 mg
Corn Seed	100~200 mg

Add 400 µL REAG2, and 60 µL REAG3, into 1.5 mL sterile centrifuge tube with plant tissue. Mix thoroughly 5-6 times upside down during the water bath (water bath: 65 °C for 30mins). For dry weight tissue, water bath time should be appropriately extended; when the sample is seed, grind with a powder grind, then treat it with liquid nitrogen.

Remove the 1.5 mL sterile centrifuge tubes from the incubate, centrifuged 5 mins at 12000 rpm and the supernate is taken to be used for next step.

96 deep well plate: Add 300 µL supernate to column 1 and column 7 of the 96 deep well plate and add 20 µL Ribonuclease A solution to column 3 and column 9. (Be aware of the column No. is for effective wells).

6 strip tube: Add 300 µL supernate to column 1 of the pre-filled and add 20 µL Ribonuclease A solution to column 3.

⚠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 *Nucleic Acid Extraction Kit.*

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

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

Note: As shown in Figure 6 and Figure 7, ensure that the 96-deep well plate and the single kit docking are properly positioned, and the marked notch of the plate faces front.

Note: Place the 96-deep well plate into the experiment cabin and push the mixing sleeves into the right position. Check the position of the mixing sleeves; otherwise, instrument dysfunction or malfunction may occur and affect the experiment results.

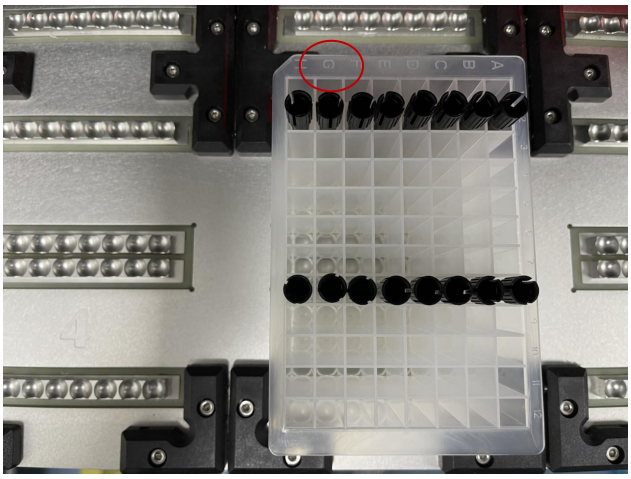

Figure 6. 96-deep well plate

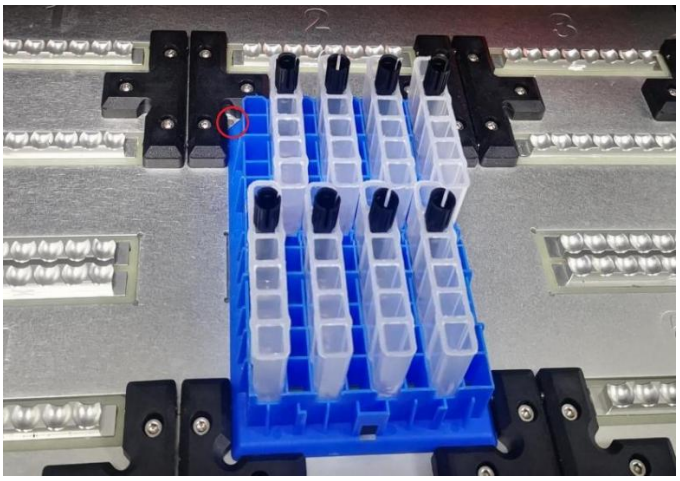

Figure 7. Put the single kit docking into the instrument

2.2.5 Procedure Run

For special operations please see 2.2.1, After the procedure is completed, the instrument will notice the user the experiment has been completed. Transfer the extracted product from column 6 and column 12 to a clean centrifuge tube which is free of nuclease. (Be aware of the column No. is for effective wells)

Note: If the user does not analyse the extracted product immediately, please seal and store in a refrigerator at -20 °C.

⚠ Caution: Any used deep well plate and mixing sleeve 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 expected results.

2.2.6 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.

Troubleshooting Guide

This troubleshooting guide should assist you in resolving any problems that arise during the experimental process. For more information, please visit our Technical Support Centre and Frequently Asked Questions,

page 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, as well as 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 press 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.	Reposition the deep well plate.
		Please make sure the mixing sleeve is inserted in place.	Reinsert the mixing sleeve.
6	Poor extraction performance	Please follow the operation requirements in the manual.	Contact the after-sales service of our company.
		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 ***Nucleic Acid 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 plant tissues samples to extract genomic DNA.

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

The extraction kit is intended for clinical diagnostics, health system and scientific research only, whose usage can act as an ancillary step for molecular detection and should be matched with other molecular detection methods. The concentration and purity of its extraction product are affected by instruments and operators. Any generated diagnostic results must be interpreted in conjunction with the other clinical or laboratory findings.

Safety Symbols and Signs

No.	Symbol	Implication
1	REF	Catalogue number
2	LOT	Batch code
3	Σ <N>	Contains sufficient for <N> tests
4		Use by date
5		Caution
6		Temperature limit
7		Reminder
8		Manufacturer
9		Do not re-use
10	CONT	Content of the Kit
11	REAG1	Pre-filled 96-deep well plate/6 strip tube
12	REAG2	Pretreatment Reagent
13	REAG3	Nucleic Acid Releaser
14		Warning
15	21 PAP	PAP21: Not-corrugated cardboard

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Contact Information

For technical assistance and more information, please contact our Technical Support Center at +86-29-826 82132 (Tel), +86-29-82216680 (Fax), inquiry@medtl.com or contact your local distributor.

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.