

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



Version 1.0

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



T822H T823H T824H



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Contents

Intended Use	1
Product Performance Indicators	1
Special Notes	1
Testing Principle	1
Content of the Kit	2
Materials Required but not Provided	2
Warnings and Precautions	2
Precautions for Safe Handling	4
Reagent Storage and Handling	4
Sample Handling and Storage	4
Operation Guide	4
1. Automated Extraction Process	4
2. Operation Steps of Automated Extraction	5
2.1 Automatic Nucleic Acid Extractor (model:GeneFlex)	5
Troubleshooting Guide	7
Quality Control	8
Limitations of Test Methods	8
Safety Symbols and Signs	8
Contact Information	٥



Intended Use

The *Nucleic Acid Extraction Kit* (*For Plant Tissues Genomic DNA Extraction-GeneFlex*) 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.

Nucleic Acid Extraction Kit (For Plant Tissues Genomic DNA Extraction-GeneFlex) 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 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* (*For Plant Tissues Genomic DNA Extraction-GeneFlex*) is worked with TIANLONG® automatic nucleic acid extractor (GeneFlex) that has 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 1-16n samples in a single run.

The *Nucleic Acid Extraction Kit* (*For Plant Tissues Genomic DNA Extraction-GeneFlex*) is used to extract genomic DNA from the samples of plant tissues(e.g. blade, pulp, seed, and tuber etc.). 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 coverall.

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 the samples of plant tissues(e.g. blade, pulp, seed, and tuber etc.).

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, 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 Nucleic Acid Extraction Kit (For Plant Tissues Genomic DNA Extraction-GeneFlex) is worked with TIANLONG® automatic nucleic acid extractor (GeneFlex). 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.

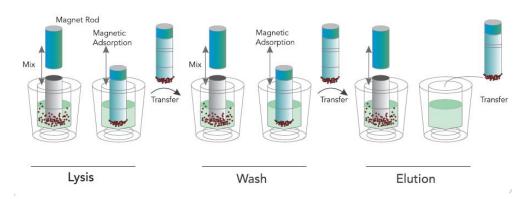


Figure 1. Schematic Diagram of Automatic Nucleic Acid Extractor

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. GeneFlex is equipped with an array of 16 magnetic rods, which allow it to process up to 16 samples simultaneously.

Content of the Kit

Name of Comp	Short Code	Т822Н	Т823Н	Т824Н
	Size	64 T/Box (Pre-filled)	20 T/Box (Pre-filled)	20 T/Box (Pre-filled)
REAG1	Component	Pre-filled 96-deep well plate	Pre-filled 96-deep well plate	Pre-filled 6 strip tube
KLAGI	Quantity	4	4	20
	Component Specification	16 Tests	5 Tests	1 Test
	Component	Pretreatment reagent	Pretreatment reagent	Pretreatment reagent
REAG2	Component Specification	25.6 mL	8 mL	8 mL
	Quantity	1	1	1
	Component	Nucleic acid releaser	Nucleic acid releaser	Nucleic acid releaser
REAG3	Component Specification	3.84 mL	1.2 mL	1.2 mL
	Quantity	1	1	1
Instructions for Use		1 Copy	1 Сору	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.

- Ribonuclease A (20 mg/mL)
- Pipettor: 20 μL, 200 μL or 1000 μL
- Tip: 20 μL, 200 μL or 1000μL
- Vortex mixer
- High-speed centrifuge
- Metal bath
- Sample holder
- 75% ethanol
- Single kit docking (matched with T824H (6 strip tube), could be purchased from Tianlong)
- Extractor

Warnings and Precautions

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

The *Nucleic Acid Extraction Kit* (*For Plant Tissues Genomic DNA Extraction-GeneFlex*) is used to extract genomic DNA from the samples of plant tissues(e.g. blade, pulp, seed, and tuber etc.). 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 the



operation. The clinical samples should be collected on a clean bench or in a bio-safety cabin.

Before using TIANLONG® automatic nucleic acid extractor (GeneFlex), 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 minutes.

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 Nucleic Acid Extraction Kit (For Plant Tissues Genomic DNA Extraction-GeneFlex) is intended for research 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 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 Nucleic Acid Extraction Kit (For Plant Tissues Genomic DNA Extraction) comes with the following warnings and precautions.

Name of Component		Hazard Classification pictograms according to (CLP) Regulation		Labelling according to Regulation	
REAG1	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	



REAG2	Pretreatment Reagent	None	None	None
REAG3	Nucleic Acid Releaser	<u>(1)</u>	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.

Precaution for Safe Handing

Do not dispose of the preparation or the packaging waste in drains leading to the sewage system or in the drainage system for waste not produced by industrial/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 (For Plant Tissues Genomic DNA Extraction-GeneFlex)* should be stored at room temperature in a cool, dry and well-ventilated area. All components of the kit can be adequately stored for 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.

Samples should be used immediately after collection to extract nucleic acid or stored at 2~8 °C for further experiment within 24 hours. 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

The automatic nucleic acid extractor (GeneFlex) enables nucleic acid extraction by magnetic beads. It uses 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. And the nucleic acid extractor performs extraction processing according to the experimental procedures. Please refer to the user manual provided with the respective instruments for operating instruction and start-up of tests.



2. Operation Steps of Automated Extraction

2.1 Automatic Nucleic Acid Extractor (model: GeneFlex)

2.1.1 Edit Experiment Program

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

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Ctor		Well	Stir	Magnetic	Wait	Speed	Volume	T Control
Step	Name	vveii	(min:s)	(min:s)	(min:s)	(rpm)	(μL)	(°C)
1	Remove bead	1	00:10	00:10	00:00	3000	630	0
2	Lysis	2	10:00	00:45	00:00	3000	700	50
3	Washing 1	3	01:00	00:30	00:00	2500	620	80
4	Washing 2	4	01:00	00:20	00:00	2500	600	80
5	Washing 3	6	00:00	00:30	00:00	2500	600	80
6	Elution	5	05:00	00:30	00:00	1500	100	80

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.

There is an electronic label attached to the left side of each deep hole plate, which can automatically recognize and extract the program on the machine.

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.

Please give priority to selecting the single base from Tianlong. Before extraction, the electronic label in the reagent needs to be attached to the left side of the base; each box is equipped with an electronic label, which can be used 20 times. Before using the 20T reagents, do not tear it off or discard the label. After using a single test reagent, a new electronic label needs to be replaced. The electronic label pasting method is shown in Figure 3.



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



Figure 3. The electronic label pasting method



2.1.3 Adding Sample to the Reagent

Sample Pretreatment

a. Take out appropriate amount of plant tissue, please refer 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	100~200mg

- b. Add 400 μ L REAG2 and 60 μ L REAG3 into 1.5 mL sterile centrifuge tube with plant tissue. In the water bath process reversed fully mixed 5-6 times (water bath: 65 °C for 30 min). For dry and weighted tissue should extension of the water bath time appropriately; when the sample is seed, grinding with a powder grinder then treated it with liquid nitrogen.
- c. Remove the 1.5 mL sterile centrifuge tubes from the incubate, centrifuged 5 min at 12000 rpm and keep the supernate for later use.
- d. Add 300 μ L supernate to the 2nd and 8th column of the 96-deep well plate (note the column no. is for effective wells), or 2nd column of the 6 strip tube. And add 20 μ L Ribonuclease A to the 3rd and 9th column of the 96-deep well plate (note the column no. is for effective wells), or 3rd column of the 6 strip tube.

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 (For Plant Tissues Genomic DNA Extraction-GeneFlex).
- 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

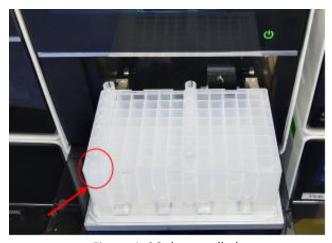
Place the 96-deep well plate or 6 strip tube in the Automatic Nucleic Acid Extractor and ensure the marked notch of the plate faces front.

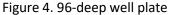
Insert the mixing sleeve into the mixing sleeve holder and close the cabin door.

Note: As shown in Figure 4 and Figure 5, 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.







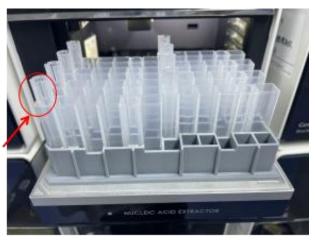


Figure 5. Put the single kit docking into the instrument

2.1.5 Experimental Procedure Run

For special operations please see 2.1.1. After the procedure completed, the instrument will notice the user the experiment has been completed. Transfer the extracted product from column 5 and column 11 to a clean centrifuge tube which is free of nuclease.

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 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.

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 (the 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 Sales Support Team 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.
_	Abnormal noise from the	The 96-deep well plate may be placed incorrectly.	Conduct re-position of the deep well plate.
instrument during extraction		The mixing sleeve may not be inserted in place.	Reinsert the mixing sleeve.
		Please follow the operation requirements in the manual.	Contact the Sales Support Team of Tianlong.
6	Poor extraction performance	The temperature control components of the instrument may be abnormal.	Contact the Sales Support Team of Tianlong.
		Others	Contact the Sales Support Team 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 (For Plant Tissues Genomic DNA Extraction-GeneFlex)** 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 the samples of plant tissues 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. Moreover, the kit uses a specially formulated eluent that can affect the absorbance value, so it is not recommended to use a UV rays spectrophotometer to measure the extraction effect directly.

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	REF	Catalogue number
2	LOT	Batch code



3	\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	Contains sufficient for <n> tests</n>
4	Σ	Use by date
5	\triangle	Caution
6	 ★	Temperature limit
7	(!)	Reminder
8		Manufacturer
9	②	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	PAP	PAP21: Not-corrugated cardboard

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.

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