TIANLONG

Nucleic Acid Extraction Kit

(For FFPE DNA Extraction, Spin-Column method)

User Guide

Σ 20 T163H

Version 5.0

CE IVD

In-Vitro Diagnostics / For use with Automatic nucleic acid extractor compatible with Nucleic Acid Extraction Kit

REF T163H

Xi'an Tianlong Science and Technology Co., Ltd.

No.389, Zhuhong Road, Xi'an, 710018, Shaanxi, P.R. China;
2-3F, No. 2 Building, Yuanzheng Innovation Park, Caotan Ecological Zone, No.1258, Hongye Road, Economic & Technological Development Zone, Xi'an, 710018, Shaanxi, P.R. China.

EC REP **SUNGO Europe B.V.**

Olympisch Stadion 24, 1076DE Amsterdam, Netherlands.

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Intended Use

The ***Nucleic Acid Extraction Kit*** is designed to extract tissue genomic DNA from paraffin-embedded (FFPE) tissue 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, Next-Generation Sequencing (NGS) 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

Extraction Yield: more than 2 μg can be extracted from 3 pieces FFPE tissue samples.

Extraction Purity: OD260/OD280≥1.5.

Special Notes

The extraction kit is particularly used for FFPE DNA isolation. Therefore, all of experiment supplies, such as pipettes, tubes, tips, must be processed by autoclave. Operator should wear gloves and masks.

Before using the kit, please read the manual and strictly follow the protocol. Clinical samples should be processed on clean bench or in bio safety cabinet.

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.

Testing Principle

During the process of nucleic acid extraction, using silicagel column principle to conduct adsorption and washing of nucleic acid, which can achieve the transfer of nucleic acid and complete the extraction and purification of nucleic acid.

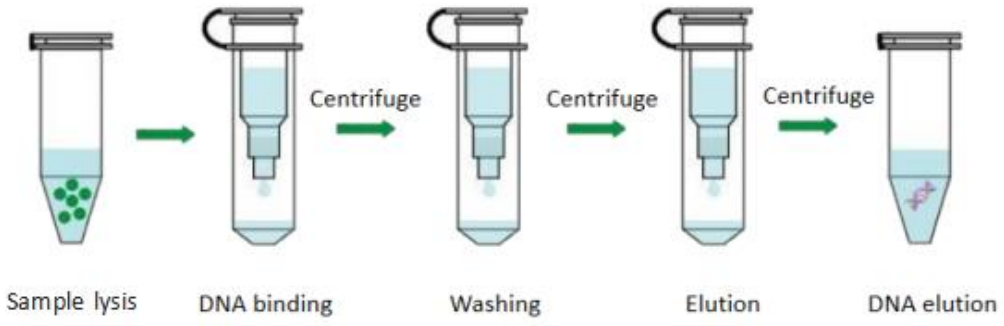

Figure 1. Schematic Diagram of Spin-Column Method

Kit Contents

NO.	Components	Specification	Quantity
1	Lysis Solution	6 mL	1 bottle
2	Washing 1	10 mL	1 bottle

3	Washing 2	10 mL	1 bottle
4	Eluent	2 mL	1 bottle
5	Proteinase K Solution	0.8 mL	1 bottle
6	Protein Digestive Buffer	4 mL	1 bottle
7	Adsorption Column	/	20
8	Collection Tube	/	20
9	Sponge Inner Support	/	1
10	Packaging Box	/	1
11	Instructions for Use	/	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: 50 µL, 200 µL, 1000 µL
- Tip: 50 µL, 200 µL, 1000 µL
- Vortex mixer
- High-speed centrifuge
- Water bath or metal bath
- Sample holder
- EtOH, xylene or TL dewaxing solution (to be ordered separately)
- 75% ethanol

Warnings and Precautions

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

The extraction kit is particularly used for FFPE 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 the operation. The clinical samples should be collected on a clean bench or in a bio-safety cabin.

In the absence of exceptional circumstances, it is prohibited to mix the reagents from different batches.

After the experiment, 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 of PDF format at https://www.ug-msds.com/MSDS1, 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 kit comes with the following warnings and precautions.

- **Product contents**

Guanidine hydrochloride, sodium dodecyl sulfate, trihydroxymethyl aminomethane, isopropanol, absolute ethanol.

- **Toxicological information**

Skin corrosion/irritation

May irritate the skin.

Severe eye damage/eye irritation

May cause irreversible eye damage.

Respiratory or dermal sensitivity

No relevant data is available.

Germ cell mutagenesis

Do not conform with the classification criteria based on the existing data.
Carcinogenicity
Do not conform with the classification criteria based on the existing data.
Reproductive toxicity
Do not conform with the classification criteria based on the existing data.
Specific target organ toxicity - single exposure
May cause drowsiness or dizziness.
Specific target organ toxicity - repeated exposure
Do not conform with the classification criteria based on the existing data.
Potential health effects
Inhalation: Avoid inhalation of concentrated vapour. Inhaling a large amount of vapour may cause respiratory irritation. May cause drowsiness or dizziness.
Skin contact: May cause skin irritation.
Eye contact: Liquid contact may cause eye damage.
Ingestion: For any unexpected route of exposure, it may be harmful if ingested.

- **Ecological Information**

Ecotoxicity: Harmful to aquatic life with long-lasting effects.
Mobility: No relevant data is available.
Bioaccumulation potential: No relevant data is available.
Environmentally adverse effects: No relevant data is available.
Other adverse effects: Do not allow the product to enter drains or water sources.

- **First aid measures**

In case of eye contact: Immediately rinse the upper and lower eyelids with plenty of water.
In case of skin contact: Immediately remove contaminated clothing thoroughly, rinse the skin with soap and plenty of water. If irritation persists, immediately contact the nearest doctor/physician.
In case of inhalation: Keep away from exposure and transfer to a place with fresh air.
In case of ingestion: Do not give anything orally to an unconscious person. Rinse mouth thoroughly with water and seek immediate medical attention for symptomatic treatment.

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

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.

Operation Guide

1. Reagent Preparation

Proteinase K Solution Preparation: The Proteinase K Solution should be stored at -20°C and not more than 6 hours at room temperature, and avoid repeated freezing and thawing (no more than 5 times).

2. Sample Pretreatment

Slice 3~5 paraffin embedded (FFPE) tissue slices (length: 0.7 cm; width: 0.7 cm; thickness 10 μM), then use the scalpel scrapes the tissue slice to get rid of the extra paraffin. (please slice the paraffin embedded (FFPE) tissue according to its size, and appropriately increases the consumption in case of the cyclic embedding organizations); Or cut a piece of the paraffin embedded (FFPE) tissue (less than 35 mg) and

place it in the precooled mortar which contains liquid nitrogen. Grind the tissue piece thoroughly while continuously adding the liquid nitrogen which can prevent the tissue from thawing. Finally transfer the grinded tissue into 1.5 mL sterile centrifuge tube and stored at room temperature, volatilize the liquid nitrogen completely.

Note: Please use internal paraffin embedded (FFPE) tissue (Slice off the external exposed tissue, since it was exposed in air for long time, the nucleic acids was seriously damaged).

3. FFPE Tissue Dewaxing

a. Add 1 mL of TL dewaxing solution or xylene (prepared by user) to a centrifuge tube containing FFPE tissue, vortex mixing and 50°C warm bath for 3 mins, this step should be operated under fume hood.

b. The 1.5 mL centrifuge tube containing the sample was centrifuged at 10000 rpm for 2 mins, then please carefully remove the supernatant dewaxing solution. In case the bottom sedimentation is not solid, please centrifuge again at 10000 rpm for 2 mins.

c. Add 1mL of absolute ethanol, vortex mixing for 10s, and then centrifuge at 10000 rpm for 2 mins, then please carefully remove the supernatant ethanol.

d. Repeat step (c) again and remove the residual ethanol as far as possible after a transient centrifugation.

e. Open the lid of centrifuge tube and leave it at room temperature for 10-15 mins, volatilize the residual ethanol completely.

4. FFPE Tissue Genomic DNA Extraction

a. The 1.5 mL centrifuge tube containing the dewaxed sample was added with 200 µL protein digestive buffer and 40 µL protease K solution respectively for tissue digestion. Cover the tube lid and water bath it at 60°C for 1h, please blend the tube several times during the water bath. Then transfer the tube to a 90°C water bath for 1h, please do not mix the tube during the water bath. Hereto the Digestion mixture is ready for use.

Note: If there are sediments in the digestive buffer, please dissolve the tube in 37°C by water bath.

b. Add 300 µL lysis solution into the centrifuge tube and mixing completely, add all the mixture into adsorption column, cover the lid and centrifuge at 10000 rpm for 1 min, discard the waste liquid in the collection tube.

c. Open the tube lid slowly, add 500 µL washing A into the adsorption column, centrifuge at 10000 rpm for 1 min and discard the waste liquid in the collection tube.

d. Open the tube lid slowly, add 500 µL washing B into the adsorption column, centrifuge at 10000 rpm for 1 min and discard the waste liquid in the collection tube.

e. Put adsorption column into the collection tube again, centrifuge at 12000 rpm for 2 mins, then put the adsorption column into a new 1.5 mL EP tube, open the lid and put in room temperature for 5 mins, until the adsorption column dry totally.

f. Add 50 µL-100 µL eluent into the adsorption column, placed that at room temperature for 5 mins, centrifuge at 10000 rpm for 1 min; then collect solution into the centrifugal tube and store it at -20°C.

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 (for 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	Poor extraction performance	Sample pretreatment	Please follow the operation requirements in the manual.
		The sample lysis may be incomplete.	Please follow the operation requirements in the manual.
		Other	Contact the after-sales service of our company.

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

Quality Control

In accordance with Tianlong Company's ISO-certified Quality Management, each lot of the ***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 paraffin-embedded (FFPE) tissue samples to purify FFPE 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.

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	Σ <N>	Contains sufficient for <N> tests
4		Use by date
5		Caution
6		Temperature limit

7	IVD	In vitro diagnostic medical device
8		Reminder
9		Manufacturer
10		Do not re-use
11	CE	Conformed with EU standard
12	EC REP	Authorized representative in the European Community

Contact Information

For technical assistance and more information, please contact our Technical Support Center at +86-29-82218051 (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.