Nucleic Acid Extraction Kit

(For FFPE DNA Extraction, Spin-Column method)

User Guide

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Version 6.0

CE IVD

REF T163H

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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 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 biosafety 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.

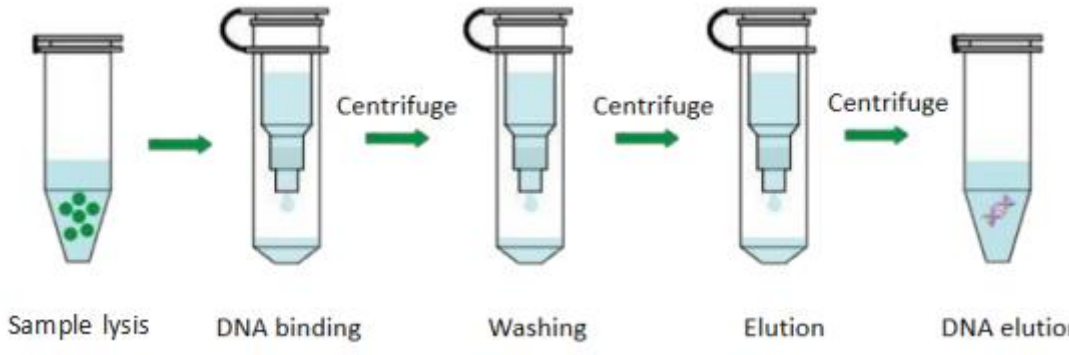

Figure 1. Schematic Diagram of Spin-Column Method

Content of the Kit

Name of Component	Component	Component Specification	Quantity
REAG1	Lysis Solution	6mL	1 bottle
REAG2	Washing 1	10mL	1 bottle
REAG3	Washing 2	10mL	1 bottle
REAG4	Eluent	2mL	1 bottle
REAG5	Proteinase K Solution	0.8mL	1 bottle
REAG6	Protein Digestive Buffer	4mL	1 bottle
REAG7	Adsorption Column	/	20
REAG8	Collection Tube	/	20
Sponge Inner Support	/	/	1
Collection Tube	/	/	1
Sponge Inner Support	/	/	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: 50µL, 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 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 kit comes with the following warnings and precautions.

Name of Component		Hazard pictograms (CLP)	Classification according to 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.
REAG2	Washing Buffer 1			
REAG3	Washing Buffer 2	None	None	None
REAG4	Elution Buffer	None	None	None
REAG5	Proteinase K Solution	None	None	None
REAG6	Protein Digestive Buffer	None	None	None

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

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.

Detail information on sample pretreatment, please refer to 2.1.3.

Operation Guide

1. 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 were seriously damaged).

2. 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 1 mL 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.

3. FFPE Tissue Genomic DNA Extraction

a. The 1.5 mL centrifuge tube containing the dewaxed sample was added with 200 µL REAG6 and 40 µL REAG5 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 1 h, 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 REAG1 into the centrifuge tube and mixing completely, add all the mixture into REAG7, cover the lid and centrifuge at 10000 rpm for 1 min, discard the waste liquid in the REAG8.

c. Open the tube lid slowly, add 500 µL REAG2 into the REAG7, centrifuge at 10000 rpm for 1 min and discard the waste liquid in the REAG8.

d. Open the tube lid slowly, add 500 µL REAG3 into the REAG7, centrifuge at 10000 rpm for 1 min and

discard the waste liquid in the REAG8.

e. Put REAG7 into the REAG8 again, centrifuge at 12000 rpm for 2 mins, then put the REAG7 into a new 1.5mL EP tube, open the lid and put in room temperature for 5 mins, until the REAG7 dry totally.

f. Add 50 µL-100 µL REAG4 into the REAG7, 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 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 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 Xi'an Tianlong Science and Technology Co., Ltd., performance evaluation studies.

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
13	CONT	Content of the kit
14	REAG1	Lysis Solution
15	REAG2	Washing 1
16	REAG3	Washing 2
17	REAG4	Eluent
18	REAG5	Proteinase K Solution
19	REAG6	Protein Digestive Buffer
20	REAG7	Adsorption Column
21	REAG8	Collection Tube
22		Warning

23		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 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.