Total DNA Extraction Kit

User Manual

Manufacturer:

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Content

Symbols

	Contains reagents for 64 reactions
	Expiration date
	*in vitro* diagnostic medical device
	Catalogue number
	Lot number
	Manufacturer
	Temperature limitation
	Date of manufacturing
	Do not reuse
	Consult the User Manual for use
	EU Authorized Representative
	Caution! Not following the instruction may lead to poor kit performance or personnel injured.

Kit Components

A. Reagents

taco ™ Total DNA Extraction Kit Cat. No.: atci-dna Number of reactions : 64		
Reagent Name	Volume	Quantity
Magnetic Bead	4 ml	1 bottle
Lysis Buffer	40 ml	1 bottle
Washing Buffer A	55 ml	2 bottles
Washing Buffer B[1]	9 ml	2 bottles
Eluting Buffer	4 ml	1 bottle
User Manual		1 copy

*Treat all reagents as potential irritants.

[1]**Add 50 ml 95% ethanol (molecular biology grade) to Washing Buffer B before use.**

Mark the label of reagent bottle after the addition of ethanol.

B. Plate & Sleeve (For single use)

Product Name	Amount (pcs)	Cat. No.
96-Well Extraction Plate	4	atcp-s
Mixing Sleeve	8	
taco™ Sticker	1	

***Do not reuse the Plate & Sleeve**

Storage & Shipping

All reagents should be stored well sealed and kept dry at room temperature (23±4°C) up to the expiration date labeled on the box. Deliver all reagents at room temperature if necessary.
Expiration dates are stated on the box and on each single component of the kit. Do not use any component of the kit beyond the expiration date. Any deviation from the instruction could influence the kit performance and must be validated by the users.

Table 1. The stability of taco Total DNA Extraction Kit

Stability	
Shelf life	1 year
Open vial stability	30 days

Concentration of Extracted DNA

The concentration of DNA depends on the sample type, number of nucleated cells in the sample, and the protocol used for isolation of DNA. Table 2 shows concentration obtained from different sample volumes and sample types.

Table 2. DNA concentration obtained from whole blood and buffy coat using taco™ Total DNA Extraction Kit

Sample type	Sample volume (μl)	Concentration (ng/μl)
Whole blood	200	≥40
Buffy coat	150	≥80

***** The normal range of white blood cell (WBC) count from fresh samples is 5000-10000 cells/ μl.

Equipment and Reagents to Be Supplied by Users

- **taco™** Nucleic Acid Automatic Extraction System (**taco™**)
- Step pipette (optional)
- Disposable gloves
- Micro-centrifuge tubes
- Micropipette (p1000, p200)
- Filter-tips (p1000, p200)
- 95% ethanol (molecular biology grade)

Introduction

The **taco™** Total DNA Extraction Kit is designed for **taco™** Nucleic Acid Automatic Extraction System. Based on the magnetic separation technology, homogenized sample cells are lysed and nucleic acids are captured by silica coated magnetic beads. Washing Buffer is then applied to remove impurities, and Eluting Buffer to recover nucleic acids from magnetic beads following serial washing steps. This kit can extract total DNA from whole blood and buffy coat. Other sample types must be validated by users.

Intended Use

The **taco™** Total DNA Extraction Kit is intended to be used for extracting total DNA from human whole blood and buffy coat for subsequent *in vitro* diagnostic application. The **taco™** Total DNA Extraction Kit is designed to be used with the **taco™** Nucleic Acid Automatic Extraction System.

This product is intended to be used by professional users such as well-trained laboratory technicians familiar with molecular biology techniques.

Any diagnostic result generated from the sample preparation procedure followed by any downstream diagnostic nucleic acid testing assay must be complemented by other clinical or laboratory finding.

Important Notes

- After receiving the kit, check the kit components for any damage. Contact GeneReach Biotechnology Corporation or your local distributor if the reagent bottles are damaged. Do not use damaged kit components since their use may lead to poor kit performance.
- Always change pipette tips between liquid transfers.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.
- Dispose of waste must be compliant to local laws.

Safety Information

To avoid injuries, when working with the kit component always wears proper clothing and accessories as recommended. For more information, please consult the appropriate Material Safety Data Sheet (MSDS).

	CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

The **Lysis Buffer** and **Washing Buffer A** contain guanidine thiocyanate, which can form highly reactive compounds when combined with bleach. Should any spillage occur, clean with suitable laboratory detergent and water.

Broken and leaky reagent buffers must be handled and discarded according to local safety regulations. Do not use damaged reagent buffers or other kit components since their use may lead to poor kit performance.

	CAUTION: Reagent buffer contains EtOH such as **Washing Buffer B** must be kept out of flame.

After the addition of EtOH to the **Washing Buffer B**, ensure the bottles are kept away from flame since it could be highly flammable, harmful and irritant.

Nucleic Acid Extraction Procedure

A. Use of taco™ Sticker

For your convenience, user may put the **taco™** Sticker on top of reagent bottles and/or on the rim of 96-Well Extraction Plate to avoid human error.

a. taco™ Sticker

- Plate Sticker:

Apply the Sticker on the rim of 96-Well Extraction Plate.

- Bottle Sticker:

Apply the Sticker on top of each reagent bottle.

b. Abbreviation Definition

LB	Lysis Buffer
M	Magnetic Bead
WA	Washing Buffer A
WAM	Washing Buffer A + Magnetic Bead
WB	Washing Buffer B
E	Eluting Buffer

B. Protocol

a. Load reagents into 96-Well Extraction Plate according to **Table 3** below at the room temperature (16-30℃) for the best kit performance.

Table 3. Loading reagent

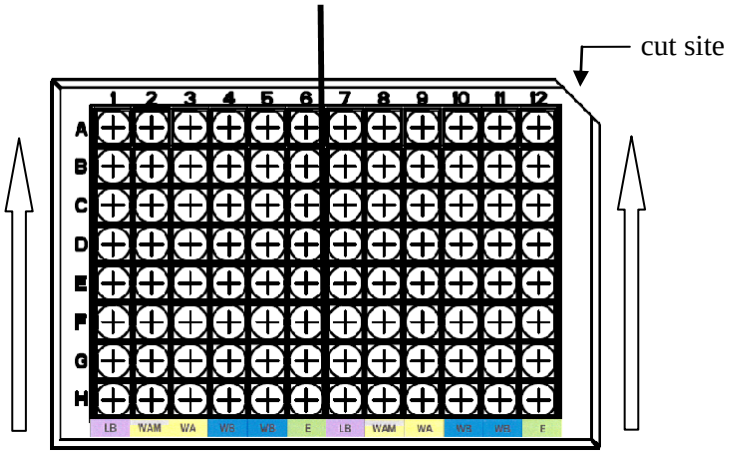
Step	Reagents
1	Add **400 µl Lysis Buffer** to **column #1 (#7)**
2	Add **750 µl Washing Buffer A** to **column #2 (# 8)**
3	Add **750 µl Washing Buffer A** to **column #3 (#9)**
4	Add **750 µl Washing Buffer B**[1] to **column #4 (#10)**
5	Add **750 µl Washing Buffer B** to **column #5 (#11)**
6	Add **50 µl Eluting Buffer** to **column #6 (#12)**
7	Add **50 µl Magnetic Bead**[2] to **column #2 (#8)**

[1] **Ensure that 50 ml 95% ethanol has been added to Washing Buffer B before the first time use.**

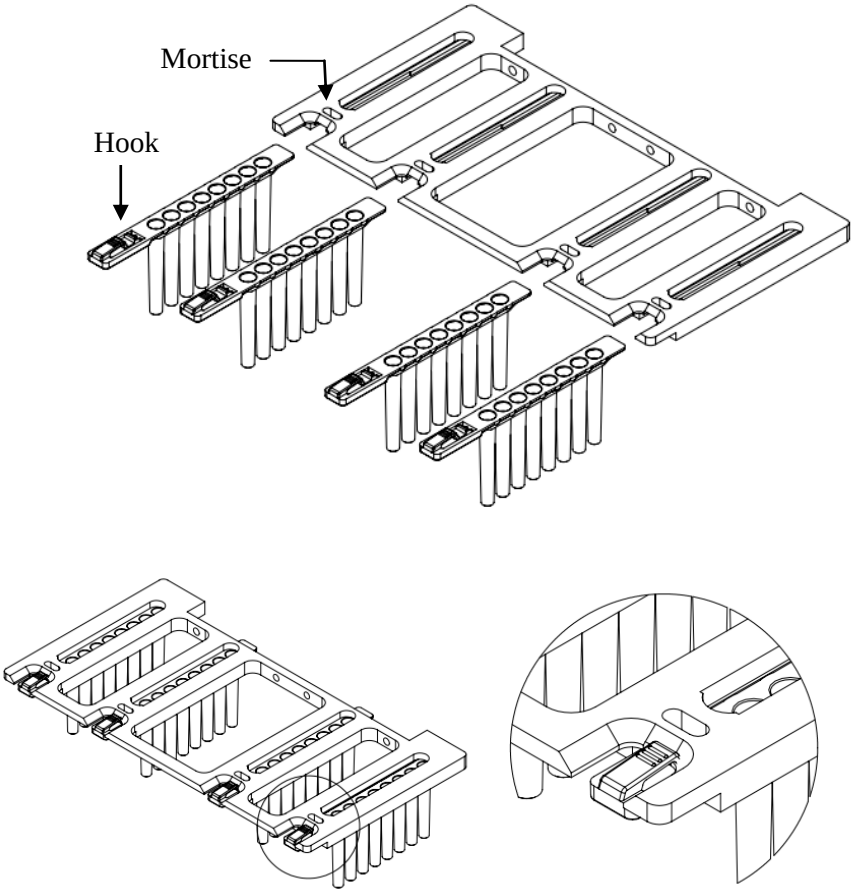
[2] **Magnetic Bead must be mixed until it's fully resuspended before each aliquot.**

b. Use micropipette to load homogenized samples into column #1 and/or #7 (See " Sample Preparation", Appendix I).

c. Open the door of **taco**^{TM} and install 96-Well Extraction Plate with reagents and samples. Push 96-Well Extraction Plate completely to the bottom of plate holder. Ensure the cut site is located on the top right.



d. Install Mixing Sleeve and lift up the Hook of Mixing Sleeve to tenon the mortise (See the illustration below).



e. Press the door button of **taco™** to close the door and press "Start" button.

f. After extraction finished, **discard the Mixing Sleeves first**.

g. **Take out the 96-Well Extraction Plate,** and press "**Reset**" button.

h. Transfer the total DNA from column #6 and/or #12 to new micro-centrifuge tubes for use (See "Purity of DNA", Appendix II).

i. It is strongly recommended to use the freshly extracted DNA for downstream applications such as amplification. Otherwise, the extracted DNA should be kept at -20℃ for longer storage (See "Storage of DNA", Appendix II).

* Do not reuse the Plate & Sleeve.

Note: Any deviation from the instruction may affect the kit performance.

Product Use Limitations

The system performance has been validated using human whole blood and buffy coat for the isolation of genomic DNA.

The kit is neither validated for use with bone marrow, cultured cells nor for the isolation of total DNA from serum, plasma, or nor for the isolation of total RNA. The user is responsible for validating the performance of the **taco™** Total DNA Extraction Kit for any particular use.

To minimize the risk of a negative impact on the diagnostic results, adequate controls for downstream applications must be used. Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

Trouble Shooting

	Comments and Suggestions
Low DNA yield	
(a) Magnetic Bead was not completely resuspended	Before starting the procedure, ensure that Magnetic Bead is fully resuspended. Vortex for at least 5 seconds before first use, and perform mild agitation before subsequent uses.
(b) Washing Buffer B did not contain ethanol	Ensure the correct volume of ethanol is added to Washing Buffer B; well seal the reagent bottle to prevent ethanol from evaporating. Add 50 ml 95% ethanol to Washing Buffer B before use. Repeat the extraction procedure with proper reagent.
(c) Reagents were loaded in wrong order	Restart the loading procedure with a new 96-Well Extraction Plate. Ensure that all reagents were loaded in the correct order and wells. Repeat the extraction procedure with new sample.

	Comments and Suggestions
(d) Low leukocyte count in the whole blood sample	Increase whole blood amount and keep the volume of buffy coat harvested constant.
(e) Poor sample quality	Using fresh sample for extraction is recommended. Poor blood quality may influence test result.
(f) Mixing Sleeves not installed	Contact your local distributor or GeneReach Biotechnology Corporation for assistance.
(g) Inappropriate operation environment	Operation temperature too high or low may lead to low yield of DNA. Ensure the operation environment of **taco™** Total DNA Extraction Kit to be performed only under room temperature (16-30˚C).
(h) Use non-recommended extraction instrument	User uses non-recommended instrument may influence the performance of **taco™** Total DNA Extraction Kit. We recommend user to apply it on **taco™**.
Poor DNA performance in downstream applications	
(a) Low volume of extracted DNA after the extraction is finished.	Repeat the extraction procedure with new sample using 100 μl Eluting Buffer.

	Comments and Suggestions
(b) Insufficient DNA used in downstream application	Quantify the extracted DNA by spectrophotometer of the absorbance at 260 nm. (See "Quantification of DNA", Appendix II)
(c) Excess DNA used in downstream application	Excess DNA can inhibit some enzymatic reactions. Quantify the extracted DNA by spectrophotometer of the absorbance at 260 nm. (See "Quantification of DNA", Appendix II)

A_{260}/A_{280} ratio for extracted DNA is low

(a) Absorbance reading at 320 nm was not subtracted from the absorbance readings at 260 nm and 280 nm	To correct the presence of Magnetic Bead particles in the eluted solution, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 nm and 280 nm.

Appendix I

A. Sample Preparation (whole blood and buffy coat)

Add 200 µl of whole blood or 150 µl buffy coat sample into column #1 (#7) of 96-Well Extraction Plate contains 400 µl of Lysis Buffer.

B. Preparation of Buffy Coat

About 10 ml of whole blood in an EDTA vacutainer tube. Prepare buffy coat by centrifuging the tube at 3000 × g for 10 minutes in room temperature. After centrifugation, 3 different layers are easy to distinguish: the upper layer is plasma; the intermediate layer is buffy coat containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes. Transfer the middle layer to a new tube carefully. It may contain small amounts of plasma and concentrated erythrocytes, aspirates off the plasma layer carefully may help harvesting the buffy coat.

Appendix II

A. Storage of DNA

Extracted DNA should be stored at 2-8°C for 24 hours or at -20°C for longer storage.

B. Quantification of DNA

The concentration of DNA should be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer.

Use Eluting Buffer as the blank to calibrate spectrophotometer. If the purified DNA needs to be diluted before the quantification, the Eluting Buffer also has to be diluted first, and the same dilution factor needs to be applied for calculation.

Collect the absorbance reading of purified DNA at 260 nm and 280 nm. The reading should fall between 0.1 and 1.0 to be accurate. An absorbance of 1 unit at 260 corresponds to 50 μg of DNA per milliliter. The ratio between the absorbance values at 260 nm and 280 nm gives an estimation of DNA purity (See "Purity of DNA").

Carryover of Magnetic Bead may affect the A_{260} reading, but should not affect the performance of DNA in downstream applications.

*Concentration of DNA sample

= 50 μg/ ml × (A_{260} − A_{320}) × dilution factor

*Total amount of DNA purified

= concentration × volume of sample in milliliters

C. Purity of DNA

Purity is determined by calculating the ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm i.e., $(A_{260}\text{-}A_{320}) / (A_{280}\text{-}A_{320})$. A subtracted absorbance reading at 320 nm is to correct the presence of Magnetic Bead particles in the eluted solution. The purity of DNA has an A_{260} / A_{280} ratio of 1.6~2.0.