Research Use Only

Total DNA Extraction Kit

User Manual

Manufacturer:

GeneReach Biotechnology Corporation

TEL: 886-4-2463-9869 Email: sales@genereach.com

No. 19, Keyuan 2nd Rd., Central Taiwan Science Park, Taichung City 407, Taiwan

Website: www.tacomag.com

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Content

Symbols


Date of manufacturing


Manufacturer

Lot number

Catalogue number


Do not reuse

Kit Components

A. Reagents

taco™ Total DNA Extraction Kit Cat. No.: atc-dna Number of reactions: 320		
Reagent Name	Volume	Quantity
Magnetic Bead	18 ml	1 bottle
Lysis Buffer	180 ml	1 bottle
Washing Buffer A	270 ml	2 bottles
Washing Buffer B[1]	40 ml	2 bottles
Eluting Buffer	55 ml	1 bottle
User Manual		1 copy

*Treat all reagents as potential irritants.

[1] Add 230 ml 95% of ethanol 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	20	atcp
Mixing Sleeve	40	
taco™ Sticker	1	

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

Storage & Shipping

All reagents should be stored well sealed and kept dry at room temperature 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 deviations from the instruction could influence the kit performance and must be validated by the users.

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

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.

Note: For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Intended Use

The **taco™** Total DNA Extraction Kit is intended to be used for extracting total DNA from whole blood and buffy coat. The **taco™** Total DNA Extraction Kit has 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 for research purpose.

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.

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 1** at the room temperature (16-30℃) for the best kit performance.

Table 1. Loading reagent

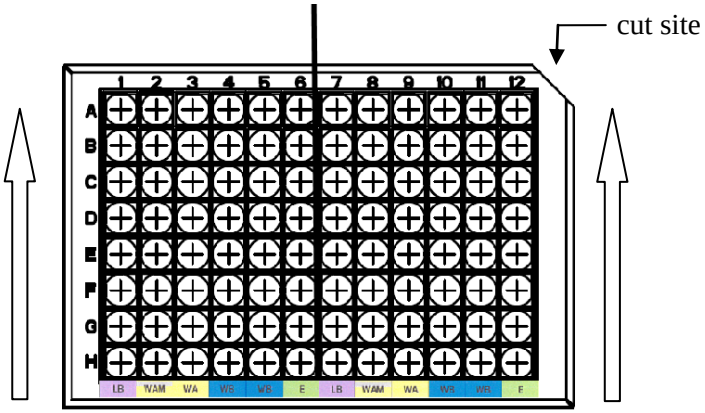
Step	Reagents
1	Add **400 µl Lysis Buffer** to **column #l (#7)**
2	Add **750 µl Washing Buffer A** to **column #2 (#8)**
3	Add **750 µl Washing Buffer A** to **column #3 (#9)**
4	Add **750 µl Washing Buffer B**[1] to **column #4 (#10)**
5	Add **750 µl Washing Buffer B** to **column #5 (#11)**
6	Add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

[1] **Ensure that 230 ml 95% ethanol has been added to Washing Buffer B before the first time use.**

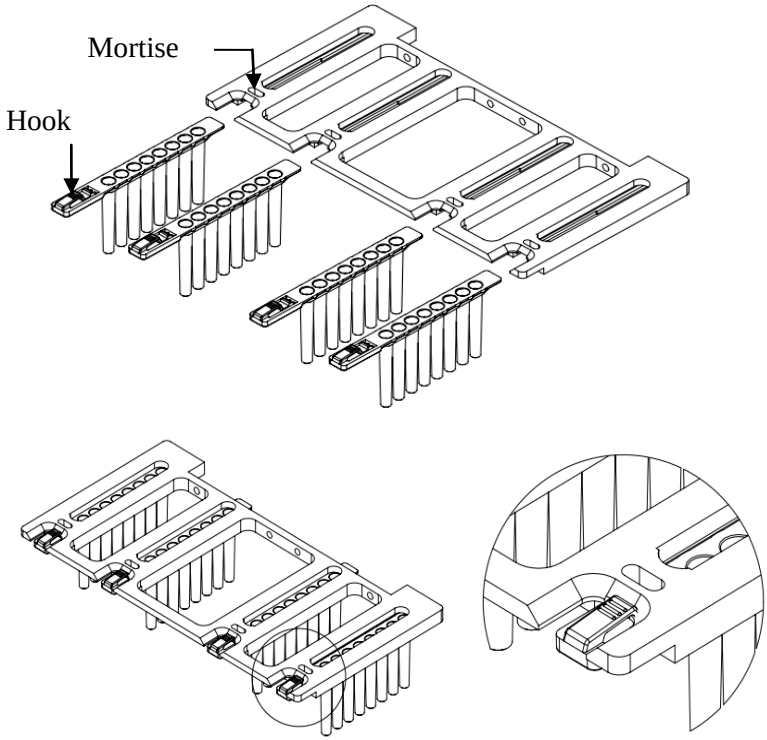
[2] **Magnetic Bead should be mixed until it's fully resuspended before each aliquot.**

b. Use micropipette (p1000) to load **homogenized samples** into **column #1 and/or #7** (See "Sample Preparation", Appendix I).

c. Open the door of **taco**™ 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 freshly extracted DNA for downstream applications such as amplification. Otherwise, the extracted DNA should be kept at -20°C for longer storage (See "Storage of DNA", Appendix II).

*Do not reuse the Plate & Sleeve.

Note: <u>Any deviation from the instruction may lead to a low yield of extracted nucleic acids.</u>

Product Use Limitations

The system performance has been validated using whole blood and buffy coat for 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.

The kit and plastic parts are not intended for any therapeutic or diagnostic of a disease for animals or humans

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 that the correct volume of ethanol is added to Washing Buffer B; well seal the reagent bottles to prevent ethanol from evaporating. Add 230 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 sample 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™**

Comments and suggestions

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 100µl Eluting Buffer.
(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 for 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 vacutainer tube. Prepare buffy coat by centrifuging the tube at 3000 x 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}-A_{320}) / (A_{280}-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.