Research Use Only

Plant DNA/RNA 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

2012/03

Content

Symbols

Date of manufacturing

Manufacturer

Lot number

Catalogue number

Do not reuse

Kit Components

A. Reagents

taco™ Plant DNA/RNA Extraction Kit Cat. No.: atc-plant Number of reactions: 320		
Reagent Name	Volume	Quantity
Magnetic Bead	18 ml	1 bottle
Lysis Buffer	200 ml	1 bottle
Washing Buffer A[1]	135 ml	2 bottles
Washing Buffer B[2]	40 ml	2 bottles
Eluting Buffer	55 ml	1 bottle
User Manual		1 copy

*Treat all reagents as potential irritants.

[1] Add 135 ml isopropanol to Washing Buffer A before use.

Mark the bottle label after adding ethanol.

[2] Add 230 ml 95% ethanol to Washing Buffer B before use.

Mark the bottle label after adding ethanol.

B. Plate & Sleeve (For one-time use only)

Product Name	Amount (pcs)	Cat. No.
96-Well Extraction Plate	20	atcp
Mixing Sleeve	40	
taco™ Sticker	2	

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

Storage

All reagents should be sealed tightly in cool and dry place at room temperature.

The expiration date of the kit and each component are stated on the label of each item. Do not use any reagent of the kit beyond the expiration date. Users should check the expiration date before use, as it could affect the accuracy of the result.

Materials and Equipments Required, but Not Provided

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

Introduction

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

Note: For research use only.

Intended Use

The **taco™** Plant DNA/RNA Extraction Kit is intended to be used for extracting nucleic acid from various plant sample types such as orchid leaf. The **taco™** Plant DNA/RNA 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 who are familiar with molecular biology techniques.

Important Notes

- After receiving the kit, please check the kit components for any damage. Contact GeneReach Biotechnology Corporation or your local distributor if reagent bottles are damaged. Do not use damaged kit, as it could affect the accuracy of the result.
- Pipette tips are all for one-time use only. Repeated usage will lead to cross-contamination.
- When working with chemicals, please always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard gloves if they are contaminated.
- Do not combine components with different batch.
- Avoid microbial contamination of the reagents.
- This kit should only be used by trained personnel.
- Disposal of waste must be compliant with local laws.

Nucleic Acid Extraction Procedure

A. Use of taco™ Sticker

For your convenience, you may put the **taco™** Sticker on top of reagent bottles and 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°C) for the best performance.

Table 1. Loading Reagent

Step	Reagents
1	Add **400 µl Isopropanol** to **column #1 (#7)**
2	Add **750 µl Washing Buffer A**[1] to **column #2 (#8)**
3	Add **750 µl Washing Buffer A** to **column #3 (#9)**
4	Add **750 µl Washing Buffer B**[2] to **column #4 (#10)**
5	Add **750 µl Washing Buffer B** to **column #5 (#11)**
6	Add **150 µl Eluting Buffer** to **column #6 (#12)**
7	Add **50 µl Magnetic Bead**[3] to **column #2 (#8)**

[1] Ensure that 135 ml isopropanol has been added to Washing Buffer A before the first time use.

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

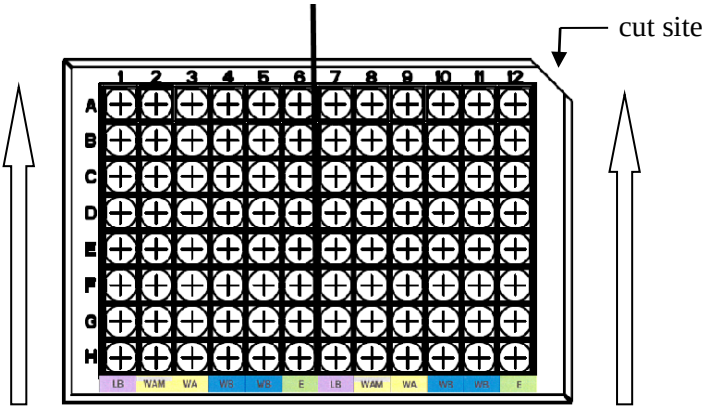
[3] Magnetic Bead must be resuspended before aliquoting.

b. Grind the plant tissue such as orchid leaf (50~150 mg) with 500-600 µl Lysis Buffer to recover at least 400 µl mixture.

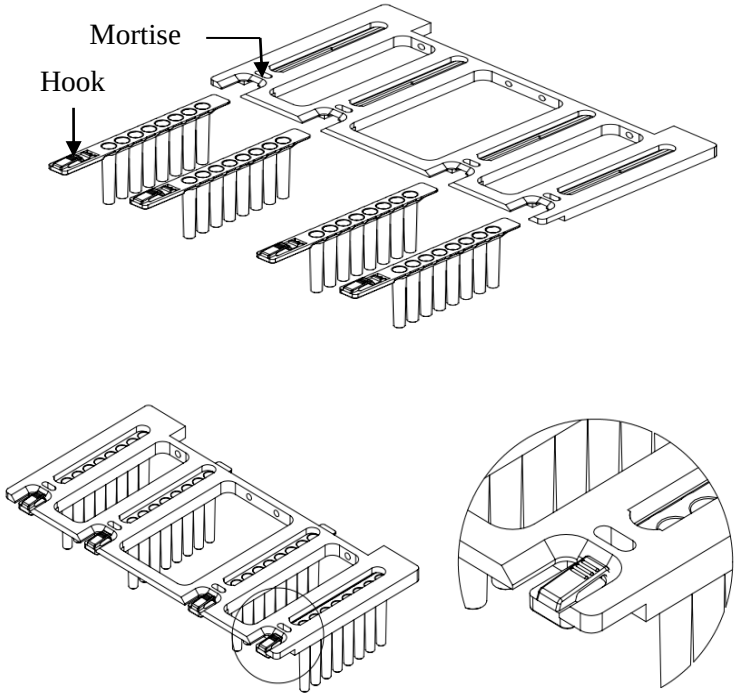
c. Vortex for 30 seconds, centrifuge at 12000 rpm for 5 minutes to spin down the debris.

d. Transfer **400 µl of the supernatant** to column #1 (#7) of the 96-Well Extraction Plate.

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



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



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

h. After the extraction procedure, **discard the Mixing Sleeves first**.

i. **Take out the 96-Well Extraction Plate,** then press "**Reset**" button.

j. Transfer the nucleic acid from column #6 and/or #12 to the new micro-centrifuge tubes for further use (See "Purity of

Nucleic Acid", Appendix II).

Note: Carryover of magnetic beads in eluates will not affect most downstream applications. If the risk of magnetic beads carryover needs to be minimized, transfer the eluates to micro-centrifuge tubes, centrifuge for 1 minute at full speed to pellet down the remaining magnetic beads, and carefully transfer the supernatants to new micro-centrifuge tubes.

k. It is strongly recommended to use freshly extracted nucleic acid for downstream applications such as amplification. Otherwise, the extracted nucleic acid should be kept at -80℃ for long-term storage (See "Storage of Nucleic Acid", Appendix II).

Note: Do not reuse the Plate & Sleeve.

Note: Any deviation from the instruction may lead to a low recovery rate of the nucleic acid extract.

Product Limitations

The system performance has been validated by using orchid leaf for nucleic acid isolation. The user is responsible for validating the performance of the **taco™** Plant DNA/RNA Extraction Kit for other particular use.

Troubleshooting

	Comments and suggestions
Low DNA/RNA yield	
(a) Magnetic Bead was not resuspended completely	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 A and B did not contain isopropanol or ethanol	Ensure the correct volume of isopropanol/ethanol is added to Washing Buffer A and B; tightly seal the reagent bottles to prevent ethanol from evaporating. Repeat the extraction procedure with proper reagent is necessary when the ethanol was not added to Washing Buffer A and B before use. (For the proper procedure of extraction, please see "Protocol").

	Comments and suggestions
(c) Reagents were loaded in wrong order	Restart the loading procedure with a new 96-Well Extraction Plate. Ensure that all reagents were loaded on the well in the correct order. Repeat the extraction procedure with new samples.
(d) Poor sample quality	Using fresh sample for extraction is recommended. Poor sample quality may influence test result.
(e) Incorrect sample volume	The kit performance would be affected if user did not use the right volume of sample. User should optimize the sample quantity when dealing with different sample types.
(f) Mixing Sleeve was not installed	Consult with GeneReach or your local distributor.
(g) Inappropriate operation environment	Operation temperature could affect the recovery rate. Please ensure the operation environment is under room temperature (16-30°C).

	Comments and suggestions
(h) Use non-recommended extraction instrument	Using non-recommended instrument may influence the performance of **taco™** Plant DNA/RNA Extraction Kit. We strongly recommend user to apply **taco™** Plant DNA/RNA Extraction Kit on **taco™**.

Poor DNA/RNA performance in downstream applications

(a) Insufficient DNA/RNA is used in downstream application	Quantify the extracted DNA/RNA by spectrophotometer of the absorbance at 260 nm. (See "Quantification of Nucleic Acid", Appendix II)
(b) Excess DNA/RNA used in downstream application	Excess DNA/RNA can inhibit some enzymatic reactions. Quantify the extracted DNA/RNA by spectrophotometer of the absorbance at 260 nm. (See "Quantification of Nucleic Acid", Appendix II)

	Comments and suggestions
Low A_{260}/A_{280} ratio	
(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. Storage of Nucleic Acid

Extracted Nucleic Acid should be stored at -80°C.

B. Quantification of Nucleic Acid

The concentration of nucleic acid should be determined by measuring the absorbance at 260 nm in a spectrophotometer.

Use Eluting Buffer as the blank to calibrate the spectrophotometer. If the purified nucleic acid needs to be diluted before the quantification, the Eluting Buffer also has to be diluted before use. Also, the same dilution factor needs to be applied for calculation.

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

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

* Concentration of nucleic acid sample

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

* Total amount of nucleic acid

= concentration × volume of sample in milliliters

C. Purity of Nucleic Acid

Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm with a background correction at 320 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. An A_{260} / A_{280} ratio of 1.6~2.2 is indicative of highly purified nucleic acid.