TANBead® Nucleic Acid Extraction Kit

Plant DNA Auto Plate

(For use with the Maelstrom 9600 series)

RUO

W613A46

(For Research Use Only) V3

1. Intended Use

TANBead® Nucleic Acid Extraction Kit (W613A46) is suitable for isolating DNA from plant samples. Automated nucleic acids extraction can be performed by Maelstrom 9600 series. Extracted nucleic acids can be analyzed by downstream applications, such as real-time PCR, next-generation sequencing.

2. Purpose

TANBead® Nucleic Acid Extraction Kit (W613A46) is suitable for extract nucleic acids of a variety of plant sample, including plant leaves and mushrooms. This kit, with Maelstrom 9600 series, enables automated nucleic acids extraction. With simple treatment of samples, it does not need repetitive centrifugations, reducing time for manual processing and lowering the risk of cross-contamination.

3. The basic principle

The silicon dioxide layer coated on the magnetic beads can adsorb the negative charged molecules to purify nucleic acids from samples.

4. Specification

Starting Materials	50 – 100 mg plant sample
Elution Volume	90 - 130 µL
Typical DNA yield	Up to 5 µg
Typical A260 / A280	≥ 1.7

5. Component Supplied with the Kit

Auto Plate	5	96 well plate with reagent buffers
96-well deep well plate (1-LB)	1	After sample preparation, 800 µL lysate will be added
Lysis Buffer	90 mL x 1	Guanidine salt, Tris buffer, surfactants
Elution Buffer	1.5 mL x 1	Nuclease-Free Water
Spin tips	96 tips	Spin Tips Assembled Box
Protocol	1	Instruction guide for user

6. Auto Plate Content and Plate Position

Plate Position	Buffer	Volume (µL)
1	Lysis Buffer	-
2	Washing Buffer 1	800 µL
3	Magnetic Beads	800 µL
4	Washing Buffer 2	800 µL
5	Washing Buffer 2	800 µL
6	Elution Buffer	130 µL
7	N/A	N/A
8	Spin Tip	-

7. Kit Storage and Shelf Life

1) Components under room temperature (15 - 35°C) can be stored until the expiration date labeled on the box.

8. Precautions

1) For research use only.
2) Avoid using expired reagents.
3) When the temperature is below 20°C, place the Auto Plates in an oven (preheated 42~60°C) for 5 to 10 minutes.
4) Avoid vigorous shaking, in order to avoid excessive formation of foam.
5) Carefully remove aluminum foil to avoid splashing.
6) Do not expose the opened reagents or Auto Plates to air. The evaporation would lead to pH change or effect on the extraction effectiveness.
7) Please check the integrity of the Auto Plates and remember to mount the spin tips into the appropriate position of the suitable instrument before operating them.
8) Please wear a mask and disposable gloves when handling.
9) Use sterile consumables to avoid nuclease contamination.
10) Reagent solution contains guanidine salt, avoid using bleach-containing detergent.
11) Avoid eyes, skin, and clothing contact with reagents. In case of any contact, flush with flowing water.
12) If any serious incident occurs, please report to the manufacturer and the competent authority of the member state in which the user and/or the patient is established.
13) Reagents are all colorless and transparent. Colored reagents indicate contamination, please replace a fresh plate before proceeding.

9. Materials required, Not Supplied

1) TANBead® Nucleic Acid Extraction System
   Model: Maelstrom 9600 series (non-sterile)
2) Disposable gloves
3) Scissors, utility knives
4) Micropipette, disposable tips (10 µL/ 200 µL/ 1000 µL)
5) 1.5 mL microcentrifuge tube
6) 15 mL / 50 mL conical tube
7) IPA: Isopropanol for molecular biology
8) CTAB buffer: 2% CTAB, 100 mM Tris pH8.0, 20 mM EDTA, 1.4 M NaCl
9) Liquid nitrogen (Optional)

10. Sample Collection, Transportation, and Storage

■ **Sample collection and storage**

1) Plant sample can be stored at
   a. RT for 24 hours
   b. 2 - 8°C up to 7 days

■ **Sample transportation**

Transportation of plant samples should follow specific plant transportation related law. Plant sample should be kept between 2 - 25°C during transportation.

11. Nucleic Acids Extraction Protocol

Sample preparation

1) Add **800 µL Lysis Buffer** to the plant sample and grind it by using the grinder or disposable pestle.
   Note: If the sample is hard to grind, it could be ground with **liquid nitrogen.** Then add **800 µL Lysis Buffer** and mix well.
2) Incubate at room temperature for **10 min**.
3) Centrifuge at **5000 – 8000 RPM** for **5 min**.

Sample preparation (with high silicon content)

1) Add **appropriate volume of CTAB Buffer** to the sample and then grind it by using the grinder or disposable pestle.
   Note: The optimal volume of CTAB buffer may be varied according to different sample types. In general, **use 4 mL CTAB buffer for 1g plant sample.**
2) Incubate at **65°C for 0.5 - 1 hour** and then centrifuged at **4000 – 6000 RPM for 5 minutes**.
3) Transfer the supernatant to a new tube and add **ice-cold IPA (0.6–1x volume of the supernatant)** and gently invert for 5–10 times.
4) Centrifuge at **6000 – 10000 RPM for 5 minutes**.
5) Discard the supernatant.
6) Add **800 µL Lysis Buffer** and mix well.

Automatic process

1) Carefully remove the aluminum foil on the Auto Plates.
2) Add **800 µL lysate** into wells of **Lysis buffer plate (1-LB)**.
3) Select the program "**613**". The parameters are given in following section.
4) Follow the guide shown on the screen and place plates carefully. Make sure that the chamfer of the reagent plate faces toward the same direction.
5) Carefully remove the Auto Plates when the program is finished.
6) Use micropipette to transfer the purified nucleic acids from **plate 6** to a clean tube.
7) Discard the used Auto Plate and spin tips into the waste recovery bin.

12. Program

■ **Maelstrom 9600**

Program Name: 613								
Plate	1	2	3	4	5	6	7	8
Volume(µl)	800	800	800	800	800	150	-	
Keep Temp.	40	40	40	-	-	30	-	
Action	For.	For.	For.	For.	For.	For.	-	
Name	LB	WB1	MB	WB2	WB2	EB	-	TIP

Step	Plate	Temp. (˚C)	Mixing (min)	Mixing (rpm)	Collect (sec)	Vapor (min)	Pause
1	3	45	1	3000	30	0	OFF
2	2	45	1	3000	30	0	OFF
3	1	45	10	3000	30	0	OFF
4	2	45	2	3000	30	0	OFF
5	3	45	2	3000	30	0	OFF
6	4	-	2	3000	30	0	OFF
7	5	-	1	3000	30	10	OFF
8	6	40	10	2700	30	0	OFF
9	6	40	0	2700	30	0	OFF
10	5	-	0.5	3000	0	0	OFF

⚠ Temperature set as “0” represents room temperature!

■ **Maelstrom 9610**

Program Name: 613								
Plate	1	2	3	4	5	6	7	8
Volume(µL)	800	800	800	800	800	150		
Keep Temp.	40	40	40	-	-	30		
Action	For.	For.	For.	For.	For.	For.		
Name	LB	WB1	MB	WB2	WB2	EB		TIP

Step	Plate	Temp. (˚C)	Mixing (min)	Mixing (rpm)	Collect (sec)	Vapor (min)	Pause
1	3	45	1	3000	30	0	OFF
2	2	45	1	3000	30	0	OFF
3	1	45	10	3000	30	0	OFF
4	2	45	2	3000	30	0	OFF
5	3	45	2	3000	30	0	OFF
6	4	-	2	3000	30	0	OFF
7	5	-	1	3000	30	10	OFF
8	6	40	10	2700	30	0	OFF
9	6	40	0	0	30	0	OFF
10	5	-	0.5	3000	0	0	OFF

⚠ Temperature set as “25” represents room temperature!

13. Reagent performance

■ **Repeatability**

Under repeatability conditions where nucleic acids are extracted with the same reagent kit on the same source samples by the same operator. The coefficient of variation of nucleic acids extraction concentration is less than 5%.

■ **Reproducibility**

A five-day reproducibility test was carried out with the same source samples for 5 consecutive days with the same reagent kit by different operators. The coefficient of variation of nucleic acids extraction concentration is less than 5%.

■ **The stability of extracted DNA**

Storage Conditions	DNA stability
-80°C	Over 90 days
-20°C	28 days
4°C	14 days
25°C	2 days
Freeze-thaw	10 times

14. Explanation of Symbols

	Manufacturer		Consult instructions for use
15°C 25°C	Temperature limit		Contains sufficient for test
REF	Catalogue number		Caution
LOT	Batch code	NON STERILE	Non-sterile
	Do not re-use		Keep away from sunlight
	Date of manufacture		Use-by date
RUO	For research use only		

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