

# **TANBead® Nucleic Acid Extraction Kit**

### Plasmid Extraction Auto Tube

M6PES46 (For Research Use Only) V2

(For use with the Maelstrom 8 series and Maelstrom 4800 series)

#### 1. Intended Use

This product is designed for isolating nucleic acid from various samples, which can be performed by using TANBead® Nucleic Acid Extractor and is intended for research use only.

### 2. Purpose

TANBead® Nucleic Acid Extraction Kit (M6PES46) is designed for a simple and convenient method of isolating high-quality plasmid DNA from  $E.\ coli$  strains including DH10B, DH5 $\alpha$ , BL21(DE3), and TOP10. This kit, with Maelstrom 8/ 4800 series, utilizes the unique non-alkaline lysis method to simplify the pre-treatment protocol of plasmid extraction. It has no repetitive centrifugation steps, reducing time for manual processing and error rate. The purified plasmid can be directly analyzed (such as Nanodrop, PCR, agarose gel electrophoresis, etc.) and used for downstream experiments.

#### 3. The basic principle

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

#### 4. Specification

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Starting Materials	E. coli culture (OD <sub>600</sub> 1 - 5)
Elution Volume	50 - 80 μL
Typical DNA yield	Up to 10 μg
Typical A260/A280	1.7 - 2.0

#### ¥96 5. Component Supplied with the Kit Auto Tube with reagent buffers Incubation Buffer 35 mL x 1 Tris buffer, surfactants Nuclease-Free Water **Elution Buffer** 1.5 mL x 1 RNase A 100 µL x 1 Add 100 µL sterile water before Lysozyme 1 2 Base A rack for 8 Auto Tubes 48 tips x 2 Spin tips Spin tip assembled box boxes Instruction manual Protocol

#### 6. Auto Tube Content

Well	Buffer	Volume (µL)
1 / 7	Binding Buffer	600
2/8	Washing Buffer 1	800
3 / 9	Washing Buffer 2	800
4 / 10	-	-
5 / 11	Magnetic Beads	800
6 / 12	Elution Buffer	80

### 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.
- The RNase A and Lysozyme are transported at room temperature.
  Upon received, please store RNase A and Lysozyme at 2-8°C.
- Incubation buffer should be stored in 4°C after the addition of Lysozyme and RNase A

### 8. Precautions

- 1) For research use only.
- 2) Avoid using expired reagents.
- 3) When the temperature is below 20°C, place the Auto Tubes in an oven (preheated 42 60°C) 5 to 10 minutes.
- 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 Tubes to air. The evaporation would lead to pH change, or effect on the extraction effectiveness.

- Please check the integrity of the Auto Tubes and remember to mount the strip 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.
- Reagent solution contains guanidine salt, avoid using bleach containing detergent.
- 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.

#### 9. Materials required, Not Supplied

- TANBead<sup>®</sup> Nucleic Acid Extraction System Model: Maelstrom 8 series, Maelstrom 4800 series (non-sterile)
- 2) Disposable gloves
- 3) Micropipette, disposable tips (10  $\mu$ L/ 200  $\mu$ L/ 1000  $\mu$ L)
- 4) 1.5 mL microcentrifuge tube
- 5) 15 mL / 50 mL conical tube

### 10. Sample Collection, Transportation, and Storage

#### ■ Sample collection

- The plasmid recovery is directly affected by host strain, antibiotic selection, culture broth, plasmid copy number, plasmid size, insert toxicity, etc. Handling the transformation and enrichment of *E. coli* properly to ensure maximum plasmid yield.
- We suggest using LB (Luria Bertani) as the culture medium for plasmid isolation. The best starting biomass of *E. coli* is an OD<sub>600</sub> of 1–5, which should be incubated overnight at 37°C.
- We do not recommend using E. coli culture during cold storage as material, it may lead to the contamination of genomic DNA.

#### 11. Nucleic Acids Extraction Protocol

- 1) Add 87.5  $\mu L$  Lysozyme and 100  $\mu L$  RNase A into Incubation Buffer
- Harvest the overnight culture of E. coli into a 1.5 mL tube by centrifugation at 6,000 × g for 2 min, then carefully remove the supernatant.
- Add 300 µL incubation buffer into a 1.5 mL tube and resuspended the pellet.
- 4) Incubate the sample at 65°C for 5 min.
- 5) Centrifuged at  $13,000 \times g$  for 3 min.
- 6) Preparing the Assembled Auto Tubes by inserting Auto Tubes into the Base completely.
- 7) Carefully remove the aluminum foil on the Auto Tubes.
- Transfer 300 μL suspension sample into well #1 / #7 of Auto Tube (Plate filled with binding buffer).
- 9) Set up spin tip:

**Maelstrom 8 series:** Hand to mount tips and make sure there is no gap between the neck of the spin tips and the spin shaft.

**Maelstrom 4800 series:** Go to Tip page and mount tips region by region.

- 10) Push Assembled Auto Tubes completely to the bottom of the plate rack. Make sure that the chamfer of the plate is at the lower left.
- 11) Select the program:

**Maelstrom 8 series**: "6PE-1" or "6PE-7" for input specimen at column #1 or column #7, respectively.

Maelstrom 4800 series: "6PE"

The parameters are given in following section.

- 12) Carefully remove the Auto Tubes when the program is finished.
- 13) Use micropipette to transfer the purified nucleic acids from well #6 / #12 to a clean tube.
- 14) Discard used Auto Tubes and spin tips into the waste recycling bin.



#### 12. Program

#### ■ Maelstrom 8 series

Program Name: 6PE-1/7						
Well	1/7	2/8	3/9	4/10	5/11	6/12
Volume	900(μL)	800(µL)	800(µL)	800(µL)	800(µL)	100(µL)

Step	Well	Action	RPM	Time (Second)	CW/CCW (Second)	Temp.	Temp. Control
1	5/11	Collection	0	30	0	0	NO
2	1/7	Mixing	2500	180	0	60	YES
3	1/7	Collection	0	30	0	60	YES
4	2/8	Mixing	3000	60	0	0	NO
5	2/8	Collection	0	30	0	0	NO
6	3/9	Mixing	3000	60	0	0	NO
7	3/9	Collection	0	30	0	0	NO
8	5/11	Mixing	3000	60	0	0	NO
9	5/11	Collection	0	30	0	0	NO
10	5/11	Vapor	0	300	0	0	NO
11	6/12	Mixing	2000	300	0	60	YES
12	6/12	Collection	0	60	0	60	YES
13	5/11	Mixing	2000	30	0	0	NO

### ■ Maelstrom 4800 series

Program Name: 6PE			Model: Maelstrom 4800 series				
Temp1	Temp2						
60	40						
Well	Name	Volume (μL)	Action	Mixing	Collect		
1/7	LB	900	For. U/D	Low	Low		
2/8	WB1	800	For.	Low	Low		
3/9	WB2	800	For.	Low	Low		
4/ 10	WB2	-	For.	Low	Low		
5/11	MB	800	For.	Low	Low		
6/ 12	EB	100	For.	Low	Low		
Step	Well	Temp (°C)	Mixing (M)	Mixing Speed (RPM)	Collect (M)	Vapor (M)	Pause
1	5	-	0	2500	0.2	0	Off
2	1	60	3	2500	0.2	0	Off
3	2	-	1	3000	0.2	0	Off
4	3	-	1	3000	0.2	0	Off
5	5	-	1	3000	0.2	5	Off
6	6	60	5	3000	1.0	0	Off

### 13. Result

Total nucleic acid yield and purity were detected using Nanodrop spectrophotometers: Up to 10  $\mu$ g DNA per test and the A260/A280 ratio of the nucleic acid is between 1.7 to 2.0.

### 14. Reagent performance

## ■ Host strain

Several *E. coli* strains including DH10B, DH5 $\alpha$ , BL21(DE3), and TOP10 can be used for plasmid isolation by this kit.

### ■ Plasmid type

Common cloning vector plasmids (such as pUC, pBS, pBR and pET) can be isolated by this kit

### Plasmid size

The plasmid which is less than < 15 kb can be isolated by this kit, isolating larger size of plasmid may decrease the yield of plasmid.

### Extraction time and throughput

Less than 29 min / 4 preps on Maelstrom 8 series Less than 27 min / 24 preps on Maelstrom 4800 series

### 15. Explanation of Symbols

***	Manufacturer	i	Consult instructions for use
15°C	Temperature limit	Σ	Contains sufficient for test
RUO	Research use only	$\triangle$	Caution
REF	Catalogue number	NON	Non-sterile
LOT	Batch code	漆	Keep away from sunlight
(2)	Do not re-use	8	Use-by date
~~	Date of manufacture		