TANBead® Nucleic Acid Extraction Kit

Gram Bacteria DNA Auto Plate

(For use with the Maelstrom 9600 series)

CE IVD

W61GA46

(For Professional Use Only) V5

1. Intended Purpose

The TANBead® Nucleic Acid Extraction Kit is a nucleic acid purification kit based on magnetic bead technology by using with corresponding TANBead® Nucleic Acid Extractor, which can automatically isolate and purify total DNA from Gram-positive,Gram-negative bacteria, or either positive or negative bacteria suchas *Mycobacterium tuberculosis*. The nucleic acid products can be analyzed directly, such as PCR, restriction digestion, PFGE (pulsed-field gel electrophoresis), etc. This kit, with TANBead® Nucleic Acid Extractor, simplifies nucleic acids extraction. With simple treatment ofsamples, it does not need repetitive centrifugations, reducing timefor manual processing, and lowering the risk of cross-contamination. The kit is intended for use by technicians, physicians, and biologists with well-trained in molecular biological techniques, the techniques of magnetic bead purification and *in vitro* diagnostic procedures. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted related to other clinical or laboratory findings. The kit is not limited to any specific disorder, condition, or other additional accompanying diagnostics. It is applicable for all population.

2. The basic principle

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

3. Specification

Starting Materials	Bacteria suspension
Elution Volume	91~130 µL
Typical DNA yield	2~5 µg
Typical A260 / A280	1.7~1.9

4. Component Supplied with the Kit

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Auto Plate	6	Auto Plate with reagent buffers
Incubation Buffer	25 mL x 1	Tris buffer, surfactants, pH 8.0
Elution Buffer	20 mL x 1	Nuclease-Free Water
Lysozyme	40 mg	Please add 1 ml Elution Buffer before using and store at -20°C
Proteinase K	1.0 mL x 1	Proteinase K
Spin tips	96 tips	Spin tip assembled box
Protocol	1	Instruction guide for user

5. Auto Plate Content and Plate Position

Plate Position	Buffer	Volume (µL)
1	Lysis Buffer	500
2	Washing Buffer 1	800
3	Magnetic Beads	800
4	Washing Buffer 2	800
5	Washing Buffer 2	800
6	Elution Buffer	130
7	N / A	N / A
8	Spin tip	-

6. Kit Storage and Shelf Life

1) Components under room temperature (15~35°C) can be stored until the expiration date labeled on the box.
2) The proteinase K is transported at room temperature. Upon receipt, please store proteinase K at 2~8°C.
3) The Lysozyme was transported at room temperature. When received, please store at -20°C.
4) Repeating freezing and thawing may cause the activity decay of Lysozyme.

7. Precautions

1) It can only be used for *in vitro* diagnostic.
2) Avoid using expired reagents.
3) When the temperature is below 20°C, place the reagent plate 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 / Auto Tubes to air. The evaporation would lead to pH change or affect the extraction effectiveness.
7) Please check the integrity of the Auto Plate / Auto Tube, 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.

8. 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

9. Sample Collection, Transportation, and Storage

■ **Sample collection and storage**

1) Bacteria can be stored at
   a. RT for 12 hours.
   b. 2~8°C up to 7 days.
   c. -80°C long-term preservation.
2) Sputum, BAL samples
   a. Samples can be collected and obtained in specific collection tubes for preservation.
   b. Follow the collection guidance of specimens you collected for routinely storage.

■ **Specimen transportation**

Transportation of bacteria specimen should follow specific bacteria transportation-related law and should be kept between 2 ~25°C during transportation.

10. Sample Pre-treatments

1) Sputum samples
   a. NaOH 1:1 mix with sputum samples for 15 minutes.
   b. Place 500 µL of the mixture into 1.5 mL vial and vortex for 30 seconds.
   c. Centrifuge the mixture at 13000 RPM for 5 minutes.
   d. Discard supernatant and the formed pellet is ready for **12.2** process.
2) BAL
   a. Vortex 30 seconds first.
   b. Place 500 µL of BAL into 1.5 mL vial and vortex for 30 seconds.
   c. Centrifuge the mixture at 13000 RPM for 5 minutes.
   d. Discard supernatant and repeat steps b to d for three times and the formed pellet is ready for **12.2** process.
3) Solid culture
   a. Place 500 µL of PBS into 1.5 mL vial and take seeding loop to take two colonies.
   b. Resuspend with vortex for 30 seconds.
   c. Centrifuge the mixture at 13000 RPM for 5 minutes.
   d. Discard supernatant and repeat steps b to d for three times and the formed pellet is ready for **12.2** process.
4) Liquid sample
   a. Follow the **12** Steps for processing.

11. Nucleic Acids Extraction Protocol

1) Centrifuge the bacterial culture at **6000 RPM** for **2 minutes**.
2) After removing the supernatant thoroughly, add **200 µL Incubation Buffer**, **10 µL Lysozyme,** and **10 µL Proteinase K**.
3) After mixing well, incubate at **56°C** for **20~30 minutes**.
4) Carefully remove the aluminum foil on the Auto Plates.
5) Transfer the lysate into wells of **plate #1** of Auto Plate (Plate filled with lysis buffer).
6) Select the program "**61G**". The parameters are given in the following section.
7) Follow the guide shown on the screen and place plates carefully. Make sure that the chamfer of the plate faces toward the same direction.
8) Carefully remove the Auto Plates when the program is finished.
9) Use a micropipette to transfer the purified nucleic acids from **plate #6** to a clean tube.
10) Discard used Auto Plates and spin tips into the waste recycling bin.

12. Program

■ **Maelstrom 9600**

Program Name: 61G								
Plate	1	2	3	4	5	6	7	8
Volume(µL)	800	800	800	800	800	150	-	-
Keep Temp.	45	30	-	-	-	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	0	0.5	3000	30	0	Off
2	2	30	0.5	3000	0	0	Off
3	1	55	20	3000	30	0	Off
4	2	30	0	3000	30	0	Off
5	1	55	10	3000	30	0	Off
6	2	30	2	3000	30	0	Off
7	3	0	2	3000	30	0	Off
8	4	-	2	3000	30	0	Off
9	5	-	2	3000	30	10	Off
10	6	30	5	3000	60	0	Off
11	5	-	0.2	3000	0	0	Off

⚠ **Temperature set as "0" represents room temperature!**

■ **Maelstrom 9610**

Program Name: 61G								
Plate	1	2	3	4	5	6	7	8
Volume (µL)	800	800	800	800	800	150	-	-
Keep Temp.	45	30	25	-	-	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	25	0.5	3000	30	0	Off
2	2	30	0.5	3000	0	0	Off
3	1	55	20	3000	30	0	Off
4	2	30	0	0	30	0	Off
5	1	55	10	3000	30	0	Off
6	2	30	2	3000	30	0	Off
7	3	25	2	3000	30	0	Off
8	4	-	2	3000	30	0	Off
9	5	-	2	3000	30	10	Off
10	6	30	5	3000	60	0	Off
11	5	-	0.2	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 acid 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 acid 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	Σ	Contains sufficient for test
CE	CE mark	IVD	In vitro diagnostic medical use
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

EC REP

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15. Post-market surveillance conclusion

After a risk assessment and clinical evaluation assessment, when weighing the benefits of medical device, patients, and the risks associated with the use of the device, the risk is acceptable. The post-market surveillance report shows that no death or serious adverse events occurred.