TANBead® Nucleic Acid Extraction Kit

Gram Bacteria Auto Plate

(For use with the SLA-16 / 32 / E13200 series)

RUO

61GA46

(For Research Use Only) V1

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 (61GA46) provides a simple and convenient method for DNA isolation from Gram-positive, Gram-negative bacteria or neither positive nor negative bacteria such as *Mycobacterium tuberculosis*. The nucleic acid products can be analyzed directly, such as PCR, restriction digestion, PFGE (pulsed-field gel electrophoresis), etc. This kit, with SLA-16 / 32 / E13200 series, simplifies 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 negatively charged molecules to purify nucleic acids from samples.

4. Specification

Starting Materials	Gram-positive / negative bacteria culture or *Mycobacterium tuberculosis* in sputum, BAL, liquid or solid culture.
Elution Volume	90~130 µL
Typical DNA yield	2~5 µg
Typical A260 / A280	1.7~1.9

5. 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	1.5 mL x 2	Nuclease-Free Water
Lysozyme	40 mg x 1	Please add 1 mL Elution Buffer before using and store at -20°C
Proteinase K	1.0 mL x 1	Proteinase K
Strip	12	8-channel strip
Protocol	1	Instruction guide for user

6. Auto Plate Content

Well	Buffer	Volume (µL)
1 / 7	Lysis Buffer	500
2 / 8	Washing Buffer 1	800
3 / 9	Magnetic Beads	800
4 / 10	Washing Buffer 2	800
5 / 11	Washing Buffer 2	800
6 / 12	Elution Buffer	130

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.
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 of freezing and thawing may cause the activity decay of Lysozyme.

8. Precautions

1) For research use only.
2) Avoid using expired reagents.
3) When the temperature is below 20°C, place the Auto Plates / Auto Tubes in an oven (preheated 42~60°C) 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 effect on the extraction effectiveness.
7) Please check the integrity of the Auto Plates / Auto Tubes and remember to insert the strips 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) The reagents are all colorless and transparent. Colored reagents indicate contamination, please replace them with a fresh plate before proceeding.

9. Materials required, Not Supplied

1) TANBead® Nucleic Acid Extraction System
   Model: SLA-16 / 32 / E13200 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

10. 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 routine storage.

■ **Specimen transportation**

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

11. Sample Pre-treatments

1) Sputum sample
   a. NaOH 1:1 mix with sputum samples for 15 minutes.
   b. Place 500 µL of the mixture into a 1.5 mL tube 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 section 12.2.
2) BAL
   a. Vortex 30 seconds first.
   b. Place 500 µL of the BAL into a 1.5 mL tube 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 extraction.
3) Solid culture
   a. Place 500 µL PBS into a 1.5 mL tube and take a 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 extraction.

4) Liquid sample
   a. Liquid samples can be used for extraction directly.

12. Nucleic Acids Extraction Protocol

Before operating, turn on the warm-up system of TANBead® Nucleic Acid Extractor, if it is equipped with temperature controller, please setting at 45°C.

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 Plate.
5) Transfer the lysate into column **#1 / #7** (column filled with lysis buffer).
6) Push Auto Plates completely to the bottom of plate rack. Make sure that the chamfer of the plate is at the lower left.
7) Push strips completely to the bottom of the strip rack frame.
8) Close the door panel.
9) Select a program "**VIRUS-W4-AUTO**". The steps are given in the following section.
10) Once the program has ended, buzzer shall alarm, take out Auto Plate carefully.
11) Use a micropipette to transfer the purified nucleic acids from column **#6 / #12** to a clean tube.
12) Discard used Auto Plate and strips.

13. Program

■ **SLA-16 / 32 series**

Program Name: VIRUS-W4-AUTO					Model: SLA-16 / 32 series			
Step	Well	Mixing (M)	Collect (S)	Rod	Mixing speed	Volume (µL)	Pause	Vapor (M)
1	3	1	60	On	Medium	800	Off	0
2	2	1	0	Off	Medium	800	Off	0
3	1	20	0	Off	Low	900	Off	0
4	2	0	60	On	Medium	800	Off	0
5	1	10	60	On	Medium	900	Off	0
6	2	2	60	On	Medium	800	Off	0
7	3	2	60	On	Medium	8000	Off	0
8	4	2	60	On	Medium	800	Off	0
9	5	2	60	On	Medium	800	Off	10
10	6	5	120	On	Medium	150	Off	0
11	5	1	0	Off	Medium	800	Off	0
12	0	0	0	Off	Medium	0	Off	0

■ **SLA-E13200 series**

Program Name: VIRUS-W4-AUTO						Model: SLA-E13200 series			
Step	Well	Temp (°C)	Mixing (M)	Collect (S)	Rod	Mixing speed	Volume (µL)	Pause	Vapor (M)
1	3	45	1	60	On	Medium	800	Off	0
2	2	45	1	0	Off	Medium	800	Off	0
3	1	45	20	0	Off	Low	900	Off	0
4	2	45	0	60	On	Medium	800	Off	0
5	1	45	10	60	On	Medium	900	Off	0
6	2	45	2	60	On	Medium	800	Off	0
7	3	45	2	60	On	Medium	8000	Off	0
8	4	45	2	60	On	Medium	800	Off	0
9	5	45	2	60	On	Medium	800	Off	10
10	6	45	5	120	On	Medium	150	Off	0
11	5	NA	1	0	Off	Medium	800	Off	0
12	0	NA	0	0	Off	Medium	0	Off	0

14. 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

15. Explanation of Symbols

	Manufacturer		Consult instructions for use
15°C 35°C	Temperature limit	Σ	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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