

TANBead® Plant DNA Auto Kit (96)

Product Code: 613046

Revised: 2011Jul

Efficiency: Nucleic acid can be extracted from plant leaf, beans, root and other plant tissue by adsorbing on the silicon dioxide surface which was coated on magnetic beads.

1. Purpose

TANBead® nucleic acid extraction kit is combined with an automatic platform for magnetic manipulation. By automatic procedure, the clean nucleic acid can be extracted from blood, plasma, serum, tissues, bacteria and cells which is provided for further molecular diagnostic applications.

2. Principle of nucleic acid purification

Paramagnetic magnetite beads were coated with a silicon dioxide layer. At an environment with high concentrations of guanidine salts, the hydroxyl groups on the surface of silicon dioxide adsorb negative charged nucleic acids. The high concentrations of guanidine salts not only promote decomposing of cell membrane, but also can surround most of proteins to separate nucleic acids and proteins.

3. Measurement of nucleic acid

It is generally measured by Ultraviolet-Visible spectroscopy and agarose gel electrophoresis after extraction of nucleic acids.

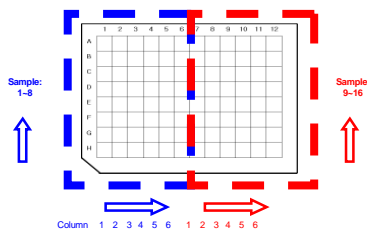
- UV spectroscopy mainly measures the wavelengths of 260nm, 280nm and 320nm. The wavelength at 260nm is the specific absorbance of nucleic acid molecules, where 280nm is the specific absorbance of protein molecules, and the measurement at 320nm is the absorbance of background disturbance caused by particles scattering. By calculating the (A260-A320)/(A280-A320) ratio, the measured purity of nucleic acids are usually between 1.75~1.85. Values less than 1.75 are considered of existing excess proteins; when larger than 1.85, it is considered to contain more amounts of RNA.
- Agarose gel electrophoresis is mainly used to measure the integrity of the extracted nucleic acids. The genomic DNA in human chromosomes is generally greater than 20kb.

4. Kit composition

- All reagents are provided for research use only.
- 16 samples can be handled at the same time with a 96 well plate.

Manual plate 96 well plate

Column	Buffer solution	Volume
1/7	Lysate	800 µl
2/8	Wash Buffer 1	800 µl
3/9	Wash Buffer 2 / Magnetic Beads	800 µl / 80 µl
4/10	Wash Buffer 3	800 µl
5/11	Wash Buffer 3	800 µl
6/12	Elution Buffer	130 µl



Content

Item	Description	Quantity
Lysis Buffer	Guanidine salt, Tris buffer, surfactants, pH 8.0	45 ml × 2
Magnetic Beads	Magnetic beads, ethanol	1.5 ml × 6
Wash Buffer 1	Guanidine salt, Tris buffer, pH 8.0.	90 ml × 1
Wash Buffer 2	Add 95 ml 95~100% ethanol before use.	5 ml × 1
Wash Buffer 3	Add 80 ml 95~100% ethanol before use.	20 ml × 2
Elution Buffer	Tris buffer, pH 8.0.	20 ml × 1
Manual	Instruction guide for user	1

NOTE: 1) CTAB buffer and IPA (isopropanol) are not included.

2) CTAB buffer: 2% CTAB, 100mM Tris pH8.0, 20mM EDTA, 1.4M NaCl

5. Notice:

A reliable extraction result depends by following the operating principles:

- Do not use expired reagents.
- Make sure to warm up reagents at room temperature for 30 minutes before use.
- Before use, inspect the completeness of the 96 well plates and strips.
- Turn on machine and go to perform program screen to warm up system for 10 minutes before using the reagent.
- All reagents should be transparent and colorless. The existence of colors indicates that the reagent is contaminated. Please replace another plate to continue following procedure.
- Please wear a mask and disposable gloves.
- Open the lid carefully to avoid splashing of the reagent solution.
- Please use sterile consumables, and make sure that they are all DNase free and RNase free.
- Replace pipette tip every time after injecting sample.
- Replace pipette tip every time before pipetting different reagent.
- Inspect all using instruments and devices to see if operations are all normal, which is to intend accuracy and correctly performing the procedures.
- The procedures may not be changed.

6. Health and safety principles

All of the reagents are only provided for using in vitro diagnostic.

- Please wear disposable gloves when operating with the reagent and samples. When finished, wash hands thoroughly.
- Since there is no known method to confirm that a contaminating source does not exist, therefore every reagent and samples used should be considered a contamination.
- Any direct contact with the samples and reagents, or even with the wash solution, should be considered a contamination.

- The samples and contaminated substances shall be deactivated before disposing. Please sterilize for 2 hours under high pressure and a high temperature at 121 °C.
- The washing of waste solutions or samples which possibly contain bioactivity shall be neutralized or sterilized before flushing into the sink.
- Because the reagent buffers contain guanidine salts, it is prohibited to wash with any detergents that contain bleach water.
- All reagents are to avoid contact with the eyes, skin, and clothes. If any contact or splashing has occurred, rinse with abundant amounts of water.

7. Storage

Storage at room temperature (15~35 °C): All reagents under room temperature can be stored to the expiration date labeled on the box.

8. Nucleic acid extraction procedure

Preparation of samples

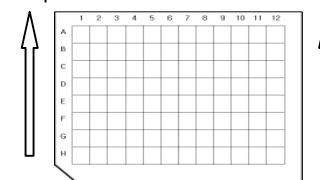
- Grind the plant tissue and **800µl lysis buffer** with grinder or disposable pestle.
- If samples are difficult to grind can be ground with **liquid nitrogen**, then add **800µl lysis buffer** and mix well.
- Incubation at **room temperature for 10min.**
- Centrifuged at **5000~8000RPM for 5min.**
- Transfer **800µl lysate**(supernatant) into **column #1/#7** of 96 well plate.

Preparation of plants with high silicon content, such as rice leaf and palm oil leaf

- Grind the plant tissue, add **CTAB buffer** and mix well. The optimal amount of CTAB buffer will change with sample. (**1g plant tissue → 4ml CTAB buffer**)
- After incubation at **65 °C for 30min~1h**, centrifuged at **4000~6000RPM for 5min** and transfer supernatant to a new tube.
- Add **cold IPA (0.6~1X lysate volume)**, invert 5~10 times and check that DNA pellet in the bottom of tube.
- Centrifuged at **6000~10000RPM for 5min.**
- Remove supernatant, add **800µl lysis buffer** and mix well.
- Transfer **800µl lysate** into **column #1/#7** of 96 well plate.

Manual plate

- Load reagent buffers into 96 well plate.
- Use micropipette to load **800µl lysate** into **column #1/#7** of 96 well plate.
- Push 96 well plate completely to the bottom of plate rack. Make sure that the missing corner of 96 well plate faces toward the door panel.



- 4) Push strips completely to the bottom of strip rack frame.
- 5) Close the door panel.
- 6) Select the program "B10-W4-AUTO". The program parameters are given in following section.
- 7) Once the program has ended, buzzer shall alarm. Turn off buzzer, and take off 96 well plate.
- 8) Use micropipette to transfer the purified nucleic acids from **column #6/#12** of 96 well plate to a clean tube.
- 9) Put the used 96 well plates and strips into the waste recovery can.
- 10) Put the used 96 well plates and strips into the waste recovery can.

The ratio of pure genomic DNA is generally between 1.75~1.85. Values less than 1.75 are considered of existing excess proteins; when larger than 1.85, it is considered to contain more amounts of RNA.

3) Electrophoresis

The 1% agarose gel electrophoresis is mainly used to measure the integrity of the extracted nucleic acid. The genomic DNA in human chromosomes is generally greater than 20kb.

Manufacture batch: As shown on box label.

Expiration date: As shown on box label.

9. Analysis of nucleic acid

1) Yield

According to ultraviolet-visible spectroscopic standard operation procedure, measure the eluate at wavelengths of 260nm, 280nm and 320nm, and adjust the absorbance between 0.1~1.0. Then the nucleic acid concentration is approximately 50 µg/ml (ng/µl) if the absorbance of A260-A320 is 1.

Example: The eluate volume: 100 µl
 A260 absorbance value: 0.9
 A320 absorbance value: 0.1
 The amount of nucleic acid is 4 µg.

$$(A260-A320) \times 50\mu\text{g/ml} \times 100\mu\text{l} \\ = (0.9-0.1) \times 50\mu\text{g/ml} \times 0.1\text{ml} = 4 \mu\text{g}$$

2) Ratio

According to ultraviolet-visible spectroscopic standard operation procedure, measure the eluate at wavelengths of 260nm, 280nm and 320nm, and adjust the absorbance between 0.1~1.0. Calculate the ratio of (A260-A320)/(A280-A320).

Example: A260 absorbance value: 0.9
 A280 absorbance value: 0.54
 A320 absorbance value: 0.1
 Therefore the ratio is 1.82

$$(A260-A320)/(A280-A320) \\ = (0.9-0.1)/(0.54-0.1) = 0.8 / 0.44 = 1.82$$

10. Program

Model : SLA-16/SLA-32				Program Name : B10-W4-AUTO					
Step	Well	Temp(°C)	Mixing (M)	Collect (S)	Rod	Mixing Speed	Volume	Pause	Vapor (M)
1	3	45	0.1	60	ON	Medium	800	OFF	0
2	2	45	0.5	60	ON	Medium	800	OFF	0
3	1	45	10	60	ON	Medium	800	OFF	0
4	2	45	2	60	ON	Medium	800	OFF	0
5	3	45	2	60	ON	Medium	800	OFF	0
6	4	45	2	60	ON	Medium	800	OFF	0
7	5	45	2	60	ON	Medium	800	OFF	10
8	6	45	10	120	ON	Medium	150	OFF	0
9	5	NA	0.1	0	OFF	Medium	800	OFF	0
10	0	NA	0	0	OFF	Medium	0	OFF	0