TANBead® Tissue RNA Auto Kit (96)

Product Code: 6K2046 Revised: 2011Nov

Efficiency: Nucleic acid can be extracted from tissues and cells 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.

a. 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 RNA is usually between 1.95~2.05. Values less than 1.90 are considered of existing excess genomic DNA.

b. 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.

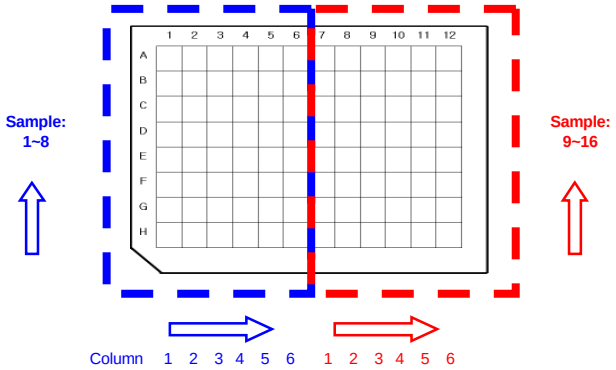
Manual Plate

96 well plate

Column	Buffer solution	Volume
1/7	Binding Buffer	300 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	100 μl

NOTE: 1) 95~!00% Ethanol of Binding Buffer was not included in the kit.

2) Elution Buffer is DEPC ddH_2O.



Content

Item	Description	Quantity
Auto Plate	The 96 well plate was pre-filled with reagent buffers.	6
Lysis Buffer	Add 450 μl β-mercaptoethanol(β-ME) before use.	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	DEPC ddH_2O	20 ml × 1
Strip	8-channel strip	12
Manual	Instruction guide for user	1

NOTE: 1) 95~100% Ethanol of Binding Buffer was not included in the kit.

2) Use RNase-free consumables.

3) If you want to remove DNA completely, please treat with DNase I. Please contact our technical service center to get more information.

5. Notice:

A reliable extraction result depends by following the operating principles:

1) Do not use expired reagents.
2) Make sure to warm up reagents at room temperature for 30 minutes before use.
3) Before use, inspect the completeness of the 96 well plates and strips.
4) Turn on machine and go to perform program screen to warm up system for 10 minutes before using the reagent.
5) 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.
6) Please wear a mask and disposable gloves.
7) Open the lid carefully to avoid splashing of the reagent solution.
8) Please use sterile consumables, and make sure that they are all DNase free and RNase free.
9) Replace pipette tip every time after injecting sample.
10) Replace pipette tip every time before pipetting different reagent.
11) Inspect all using instruments and devices to see if operations are all normal, which is to intend accuracy and correctly performing the procedures.
12) The procedures may not be changed.

6. Health and safety principles

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

1) Please wear disposable gloves when operating with the reagent and samples. When finished, wash hands thoroughly.
2) 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.
3) Any direct contact with the samples and reagents, or even with the wash solution, should be considered a contamination.
4) The samples and contaminated substances shall be deactived before disposing. Please sterilize for 2 hours under high pressure and a high temperature at 121℃.
5) The washing of waste solutions or samples which possibly contain bioactivity shall be neutralized or sterilized before flushing into the sink.
6) Because the reagent buffers contain guanidine salts, it is prohibited to wash with any detergents that contain bleach water.
7) 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℃): All reagents under room temperature can be stored to the expiration date labeled on the box.

8. Nucleic acid extraction procedure

Preparation of samples

For cell (2~5 ×10^5 cells / 500μl lysis buffer)

1) Cultured cells are centrifuged at **3000RPM/10min** → remove supernatant thoroughly
2) Add **500μl lysis buffer** → mix well by pipetting(very important) → incubation **at RT. for 10min**

NOTE: Binding Buffer : Lysate = 300 : 500

For tissue (30~50mg tissues / 800μl lysis buffer)

1) Load tissues and **800μl lysis buffer** into TissueLyser.
2) Mix well and stand for 10 minutes at room temperature.
3) Centrifuged at 6000RPM for 5 min.
4) After centrifugation, transfer **500μl lysate** into **column #1/#7** of 96 well plate.

NOTE: Binding Buffer : Lysate = 300 : 500

For shrimp sample

Size of shrimp	Sample source	Sample size
10~30g	gill	one piece
	pleopod	one leg
	hepatopancreas	~10mm^3
2~10g	gill	few pieces
	pleopod	few leg
	hepatopancreas	~10mm^3

1) Add shrimp sample into **V shape tube** with **800μl lysis buffer** and grind well with a disposable grinder.
2) Incubated at room temperature for 5~10min.
3) Centrifuged at 6000RPM for 5 min.
4) After centrifugation, transfer **500μl of the upper clear solution** into **column #1/#7** of 96 well plate.

NOTE: Binding Buffer : Lysate = 300 : 500

Manual plate

1) Load reagent buffers into 96 well plate and transfer **500μl lysate** into **column #1/#7** of 96 well plate.

NOTE: 1) 95~100% Ethanol of Binding Buffer was not included in the kit.

2) Binding Buffer : Lysate = 300 : 500

1) 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.
2) Push strips completely to the bottom of strip rack frame.
3) Close the door panel.
4) Select the program "**B10-W4-AUTO**". The program parameters are given in following section.
5) Once the program has ended, buzzer shall alarm. Turn off buzzer, and take off 96 well plate.
6) Use micropipette to transfer the purified nucleic acids from **column #6/#12** of 96 well plate to a clean tube.
7) Put the used 96 well plates and strips into the waste recovery can.

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 RNA concentration is approximately 40 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 3.2 μg.

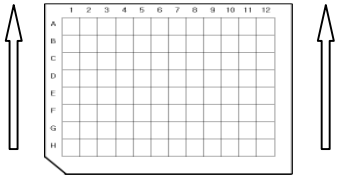
(A260-A320) × 40μg/ml × 100μl
= (0.9-0.1) × 40μg/ml × 0.1ml = 3.2 μg

1) 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: 0.50
A320 absorbance value: 0.1
Therefore the ratio is 2.00

(A260-A320)/(A280-A320)
= (0.9-0.1)/(0.50-0.1) = 0.8 / 0.40 = 2.00



❒ The ratio of pure RNA is generally between 1.95~2.05. Values less than 1.90 are considered of existing excess genomic DNA.

3) Electrophoresis

The 1.2% agarose gel electrophoresis is mainly used to measure the integrity of the extracted RNA. 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.

10. Program

Model：SLA-16/SLA-32				**Program Name：B10-W4-AUTO**					
Step	**Well**	**Temp(℃)**	**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