Step	Well	Name	Waiting Time （min ： ss）	Mixing Time （min ： ss）	Magnet Time （min ： ss）	Adsorption	Speed	Volume （μL）
1	1	Mixing	0 : 0	0 : 30	0 : 0		F	900
2	6	Beads	0 : 0	0 : 15	0 : 30	√	M	200
3	1	Binding	0 : 0	10 : 0	0 : 35	√	F	900
4	2	Wash 1	0 : 0	2 : 0	0 : 30	√	F	600
5	3	Wash 2	0 : 0	1 : 0	0 : 30	√	F	600
6	4	Wash 3	0 : 0	1 : 0	0 : 30	√	F	600
7	5	Elution	1 : 0	5 : 0	1 : 00		M	100
8	6	Discard	0 : 0	0 : 30	0 : 0		M	200

Analysis Nucleic Acid

Get some RNA,diluted in a advisable factor with elution buffer.

Survey the OD260, OD280 and OD320.

Concentration（μg/mL）＝40×OD260×dilution fact

2.1≥OD260-320/ OD280-320≥1.7

Notice: 1.0≥OD260≥0.1, the result of ratio is much reliable.

Company Information

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MagaBio plus Total RNA Purification Kit II

Kit Components

Cat#	BSC69T1E	BSC69S1E	BSC69S1B	BSC69M1B
Components	**16 tests**	**32 tests**	**50 tests**	**100 tests**
Lysis Buffer	11.2 mL	22.4 mL	35 mL	35 mL×2
Binding Buffer	96 well pre-packed plate 2 pieces	96 well pre-packed plate 2 pieces	30 mL	60 mL
DNase Stop Buffer			12.6 mL (add 18.9 mL absolute ethanol before use)	25.2 mL (add 37.8 mL absolute ethanol before use)
Wash Buffer			18.9 mL (add 44.1 mL absolute ethanol before use)	37.8 mL (add 88.2 mL absolute ethanol before use)
RElution Buffer			10 mL	20 mL
MagaBio Reagent			0.75 mL	0.75 mL×2
Handbook V1.0	1	1	1	1

Storage

- The kit can be transported at room temperature.
- Kit storage: lysis buffer was stored at 2-8℃ and other components were stored at room temperature.
- All reagents, when stored properly, are stable for 12 months from the time of delivery.

Introduction

The kit provides a very simple, fast and cost effective technique to isolate high quality RNA. Using one simple protocol, high yield of purified RNA can be isolated from various sources including blood, culture cells,leukocytes,tissue and plant. MagaBio sample processing is based on proprietary magnetizable particles--MagaBio. The pure RNA can be applied extensively in RT-PCR, Northern hybridization, mutant analysis, RNAi and the others.

According to the special interaction, use MagaBio nucleic acid separation system with a general protocol---sample processing, MagaBio adsorption, washing and elution, and can go high-throughput.

Principle and Advantage

RNA in the sample is liberated using Lysis Buffer. Released RNA is bound exclusively and specifically to the MagaBio Reagent in presence of Binding Buffer. The RNA bound to MagaBio particles is captured by the magnetic material and contaminants are removed by washing with DNase Stop Buffer and Wash Buffer. The RNA is then eluted from the particles with Elution

Buffer or molecular grade water.

Apparatus and materials to be prepared by the user

* Magnetic Rack * Water bath or Dry bath
* Vortex mixer * Absolute alcohol (For BSC69S1B and BSC69M1B)

Important Notes

- The following procedures are applicable to the Bioer NPA-32P purification instrument. If use other purification instrument, the operating procedures shall be adjusted according to the performance of different instruments.
- MagaBio Reagent should be mixed well (particles are uniformLy suspended) every time.
- Proper microbiological, aseptic technique should always be followed when working with RNA. All plasticware and glassware should be free of RNase contamination. Keep the tubes closed during the procedure, work quickly, and keep the purified RNA on ice until used.

Protocol

The manual purification

1．Sample Lysis processing

1) Animal tissue: Add 700μL of well mixed Lysis Buffer to the 1.5mL centrifuge tube.Grind the tissue into powder under the liquid nitrogen. No more than 30mg tissue will be add to the tube above. Mix thoroughly. Incubate at room temperature for 5 minutes. Or add 700μL of well mixed Lysis Buffer to the Grinding tube. No more than 30mg tissue will be add to the tube above. Grinded by grinder . Incubate at room temperature for 5 minutes.

2) Culture cells :(attached cells should be digested by trypsin) spin in 1,000×g for 2min, discard the supernate. No more than 10^7 cells. Add 700μL of well mixed Lysis Buffer, mix well by pulse-vortexing until a homogeneous mixture is obtained, usually 30 seconds. Incubate at room temperature for 5 minutes.

3) Whole blood: Add 200μL sample into the 1.5mL centrifuge tube. Then add 600μL of well mixed Lysis Buffer to the microcentrifuge tube. Mix well by pulse-vortexing until a homogeneous mixture is obtained, usually 30 seconds. Incubate at room temperature for 5 minutes.When the sample volume is great than 200μL,treated with Red Blood Cell Lysis Buffer Kit (Cat. # BSA06M1),then follow the leukocyte extraction procedure.

4) Leukocytes: The whole blood was extracted and centrifuged as soon as possible , centrifuge for 10 minutes at 3000rpm, drain the buffy coat, or prepare white blood cells fresh whole blood according to standard procedures of Red Blood Cell Lysis Buffer Kit (Cat. # BSA06M1 or Cat. # BSA07M1). Add 700μL Lysis Buffer to the 1.5mL centrifuge tube. Vortexing for 30s. Incubate at room temperature for 5 minutes.

2．Chloroform extraction

Add 140μL chloroform to the pyrolysis product above. Mix thoroughly. Incubate at room temperature for 2-3 minutes, centrifuge for 10min at the speed of 13000g. After centrifugal, the mixture stratification: supernatant layer, interlayer, substrate; The RNA exists in supernatant layer. Transfer 300-400μL supernatant layer to a clean centrifuge tube.**(Note: The volume of the supernatant layer no more than 400μL)**

3．Add Binding Buffer

Add 600μL Binding Buffer in tube.

4．MagaBio adsorption

1) Add 15μL MagaBio Reagent in tube.

2) Mix the tube gently and incubate for 10 minutes at room temperature, while mixing.

Note: using an end-over-end rotator or manual mixing every 2-3 minutes.

3) Sediment the MagaBio RNA bound particles using a magnetic rack. Aspirate the supernate, remove the tube from the magnetic rack and wash the particles as described in below.

5．Washing

1) Add 600 μL of DNase Stop Buffer to the tube above. Mix well by inverting the tube several times to ensure the particles are completely dispersed. Sediment the particles on the magnetic rack and aspirate the supernatant.

2) Add 600 μL of Wash Buffer to the tube from the above. Mix well by inverting the tube several times to ensure the particles are completely dispersed. Sediment the particles on the magnetic rack and aspirate the supernatant.

3) Remove the tube from the magnetic rack and repeat washing once more following the step2.

4) Short centrifugation, use 10μL pipette to absorb the residual liquid.

6．Elution

1) Add 50~100μL of RElution Buffer and mix for 5 minutes.

2) Sediment the particles on the magnetic rack and carefully transfer the supernatant containing the isolated RNA into a clean tube. The material is ready for further analysis. If the isolated RNA sample is not going to be tested on the same day, freeze at -20℃ until the time of analysis.

The automation purification

An example for applying the kit on our product NPA-32P:

1．Reagent prepare

1) For BSC69S1B and BSC69M1B

Add 600μL Binding Buffer to the 2.2mL 96 Deep Well column 1 and 7; 600μL DNase Stop Buffer to the column 2、8,600μL Wash Buffer to the column 3、4 and 9、10,100μL Elution Buffer to the column 5、11, 185μL pure water and 15μL MagaBio Reagent to the column 6、12.

2) For BSC69T1E and BSC69S1E

Shake 96-well plate upside down for three times after placing under room temperature, then rip off plastic film, centrifuge in 96-well centrifuge for a couple of seconds(or swing by hand) to avoid adhered liquid. Rip off aluminum foil film of 96 well plate, identify the direction of the plate (magnetic beads in column 6th&12th).

2．The sample lysis processing and chloroform extraction are the same with the manual extraction.

3．Transfer 300-400μL supernatant layer to the 96 deep well column 1、7.

4．Place 96 deep well to the instrument, then plug in 8-strip Tip and run the program.