

MagaBio plus Total RNA Purification Kit

Kit Components

Cat#	BSC53T1E	BSC53S1E	BSC53S1B	BSC53M1B
Components	16 tests	32 tests	50 tests	100 tests
ML Buffer	6.4 mL	12.8 mL	20 mL	40 mL
Binding Buffer	96 well pre-packed plate 2 piece	96 well pre-packed plate 2 pieces	30 mL	60 mL
DNase Stop Buffer			12.6mL (add 18.9mL absolute ethanol before use)	25.2 mL (add 37.8mL absolute ethanol before use)
Wash Buffer			18.9mL (add 44.1mL absolute ethanol before use)	18.9mL×2 (add 44.1mL absolute ethanol before use)
RElution Buffer			10 mL	20 mL
MagaBio Reagent			0.5 mL	1 mL
Handbook V1.0	1	1	1	1

Storage

- ◆ The kit can be transported at room temperature.
- ◆ All reagents are 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 ML 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.

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# MagaBio plus Total RNA Purification Kit



## Apparatus and materials to be prepared by the user

- \* Magnetic Rack
- \* Water bath or Dry bath
- \* Vortex mixer
- \* Absolute alcohol (For BSC53S1B and BSC53M1B)

## 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.
- If the room temperature is too low, we can put the ML Buffer at 56°C warm bath about 10min for preheating.
- 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) Whole blood:** Add 200μL sample into the 1.5mL centrifuge tube. Then add the same volume of ML Buffer to the microcentrifuge tube. Mix well by pulse-vortexing until a homogeneous mixture is obtained, usually 30 seconds. Incubate at room temperature for 10 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.

**2) 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). Resuspend the Leukocytes in 200μL of PBS. Mix gently to break the pellet into a completely uniform suspension. Add 200μL ML Buffer to the 1.5mL centrifuge tube. Vortexing for 30s. Incubate at room temperature for 10 minutes.

**3) Animal tissue:** Add 400μL of well mixed ML Buffer to the 1.5mL centrifuge tube. Grind the tissue into powder under the liquid nitrogen. No more than 25mg tissue will be added to the tube above. Mix thoroughly. Incubate at room temperature for 10 minutes. Centrifuge for 5min at the speed of 13000g. Carefully transfer the supernatant into a clear 1.5mL centrifuge tube.

**4) Plant tissue:** Add 400μL of well mixed ML Buffer to the 1.5mL centrifuge tube. Grind the tissue into powder under the liquid nitrogen. No more than 50mg tissue will be added to the tube above. Mix thoroughly. Incubate at room temperature for 10 minutes. Centrifuge for 10min at the speed of 13000g. Carefully transfer the supernatant into a clear 1.5mL centrifuge tube.

**5) Culture cells :** (attached cells should be digested by trypsin) spin in 3000 rpm for 2min, discard the supernate. No more than 10<sup>6</sup> cells. Add 400μL of well mixed ML Buffer, mix well by pulse-vortexing until a homogeneous mixture is obtained, usually 30 seconds. Incubate at room temperature for 10 minutes.

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## 2 · Add binding buffer

Add 600µL Binding Buffer in tube.

## 3 · MagaBio adsorption

1) Add 10µL 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.

## 4 · 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.

## 5 · 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°C until the time of analysis.

## The automation purification

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

### 1 · Reagent prepare

1) For BSC53S1B and BSC53M1B

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, 190µL pure water and 10µL MagaBio Reagent to the column 6、12.

2) For BSC53T1E and BSC53S1E

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 of samples are the same with the manual extraction.

3 · Add the lysate (Tissue sample: Add the supernate after lysis) 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.

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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	0 : 30		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.

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