containing the flow-through. Add 30–100 μL RElution Buffer to the center of the membrane, incubate for 2 min at room temperature, and then centrifuge at 12,000g for 2 min. If not use immediately, please store at -80℃.

【Troubleshooting】

Q1: Low yield or no RNA

A1： None or incorrect ethanol add to Wash Buffer II. And the PK Solution should be stored at 2 ℃-8℃.

Q2: Worry about dumping of liquid in the collection tube can cause cross contamination.

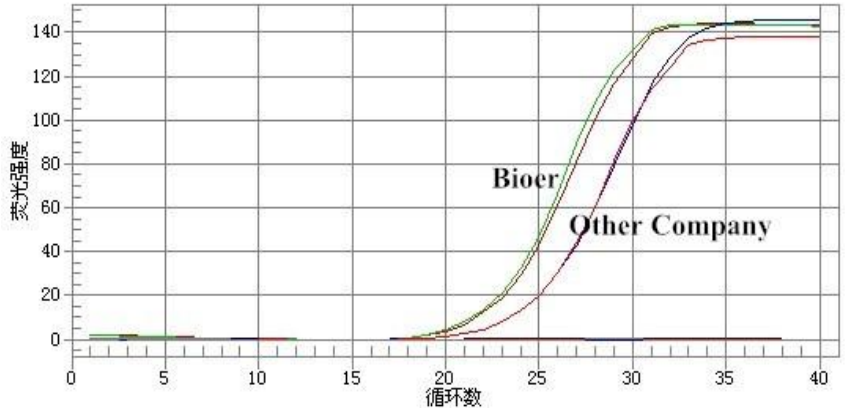
A2: Can buy separate the collection tube, each time change.

Q3: Genome DNA residues in the extraction of RNA

A3： The DNase I should be stored at -20 ℃.

【Analysis RNA】

Real-Time RT-PCR compare



Biospin FFPE Tissue RNA Extraction Kit

BSC66S1

TECHNICAL SUPPORT:

For technical support, please dial phone number ： 0086-571-87774567-5287 or 5297, or fax to 0086-571-87774553

Email to reagent@bioer.com.cn.

Website: www.bioer.com.cn

【Kit Components (50T)】

Cat# BSC66S1	
Component	**Amount**
Deparaffinization Solution	50mL
Lysis II Buffer	10mL
Binding Buffer	11mL
SW Buffer	26mL
Wash Buffer II	8mL（Add 32 mL ethanol before use）
RElution Buffer	10mL
PK Solution	500μL (Stored at 2-8℃)
Spin Column	50
Handbook	1
Cat# BSA35S2 (Stored at -20℃)	
Component	**Amount**
DNase I Buffer	2.6 mL
DNase I	100μL

【Storage and transportation】

- The Kit has demonstrated stability of 12 months when the PK Solution should be stored at 2-8℃,DNase I should be stored at -20℃，others at room temperature.
- The kit can be transported at room temperature, but PK Solution and DNase I need cold chain.

【Introduction】

This kit is used to extract high- purity RNA from FFPE tissue sections, with non-toxic deparaffinization solution, high-performance Lysis II Buffer to release RNA from FFPE efficiently.The high efficient binding of RNA to our spin matrix while proteins and other impurities are removed by wash buffer. Nucleic acids are easily eluted with sterile RNase free water or RElution Buffer. The purified RNA is ready for downstream applications such as Real-Time PCR, Sec.

【Apparatus and materials to be prepared by the user】

* 1.5mL microcentrifuge tubes * 10μL/100μL/1000μL tips
* centrifuge capable of ≥14,000g * ethanol（≥95%）
* Heating block or water bath

【Important note】

1）Wash Buffer II: Add the ethanol as the volume marked on bottle label and mix well.
2）Lysis II Buffer precipitates below room temperature. It is critical to warm up the buffer at 50℃ to dissolve the precipitate before use.
3）PK Solution should be stored at 2℃-8℃.
4）DNase I should be stored at -20℃.
5）Carry out all centrifugations at room temperature.

【Procedure】

1. Sample processing

a）FFPE sections with a thickness of up to 10 μm. Up to 8 sections, each with a thickness of up to 10 μm and a surface area of up to 5×5 mm, placed in a 1.5mL microcentrifuge tube.

b）Paraffin-embedded blocks: Use a sterile scalpel to cut off the paraffin surface in contact with air, scrape up to 30mg of sample, Avoid paraffin as possible, placed in a 1.5mL microcentrifuge tube.

c）Formalin fixed tissue samples: Cut the sample to small pieces, placed in a 1.5mL microcentrifuge tube. Add 1mL 10mM pH7.0~7.4 PBS or physiological saline, mix with vortex, centrifuge at full speed for 1 min, Remove the supernatant by pipetting. Repeat this step again, and then start step 5.

2. Add 1mL Deparaffinization Solution to the 1.5mL tube, close the tube and vortex 10s,place the tube in heating block or water bath at 56℃ for 3 min.
3. Centrifuge at 10,000g for 1 min, and remove the supernatant by pipetting.
4. Add 1m ethanol to the tube, and mix 10s by vortexing. Centrifuge at 10,000g for 1 min, Remove the supernatant by pipetting, Open the tube and incubate at room temperature or up to 37℃. Incubate for 10 min or until all residual ethanol has evaporated.
5. Add 200μL Lysis II Buffer,10μL PK Solution to the tube，mixed by vortexing, Then Incubate at 56℃ for 15 min .
6. Incubate at 80℃ for 15 min.
7. Centrifuge at 10,000g for 2 min., Transfer the supernatant to new 1.5 mL tube. Add 220μL binding Buffer mixed by vortexing.
8. Add 660μL ethanol and mixed by vortexing.
9. Transfer 700μL mixture to Spin column, centrifuge at 10,000g for 1 min. Discard the flow-through.
10. Transfer the remaining mixture to Spin column, centrifuge at 10,000g for 1 min. Discard the flow-through.
11. Add the mixture of 48μL DNase Buffer and 2μL DNaseI into the spin column, Incubate in room temperature for 15min.
12. Add 500μL SW Buffer to the spin column, centrifuge at 10,000g for 1 min. Discard the flow-through.
13. Add 500μL Wash Buffer II (ethanol added) to the spin column; centrifuge at 10,000g for 1 min. Discard the flow-through.
14. Add 200μL Wash Buffer II (ethanol added) to the spin column; centrifuge at 10,000g for 2min. Discard the flow-through.
15. Place the column in a clean 1.5 mL micro centrifuge tube, and discard the collection tube