BIOER

Kit Components (100T)

Component	Amount
RLysis Buffer	17.5ml
RD Buffer	35ml
DNase Buffer	4ml
$MnCl_2$	900ul
DNase I	100ul(stored at -20℃)
DNase Stop Buffer	10 ml (add 13 ml ethanol before use)
Wash Buffer	32 ml(add 48ml ethanol before use)×2
RElution Buffer	20ml
Spin columns	100
Handbook	1 copy

Storage and transportation

- The Kit has demonstrated stability of 18 months when the DNase I should be stored at -20 ℃, others at room temperature.
- The kit can be transported at room temperature.

Introduction

The kit is a ready-to-use reagent for the isolation of total RNA from animal tissues, cells, bacterias and anothers (plant tissues are not recommended). Add RLysis Buffer to the processed sample.RD buffer will remove the protein,then adding alcohol will bind RNA to spin column. The DNA will be destroied by the DNase I reaction. Then RNA can be easily isolated through several washing and eluting steps.

The kit provides a very simple, fast and economical technique to isolate high quality RNA, and can go high-throughput. The pure RNA can be applied extensively in Northern blot, blotting hybridization,in vitro translation,RNase protect assay, RT-PCR/Real time RT-PCR analysis,construction cDNA library etc.

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Apparatus and materials to be prepared by the user

* Sterile 1.5ml centrifuge tubes
* microcentrifuge capable of 14,000rpm
* Vortex mixer
* 10μl/100μl/1000μl tips
* Absolute alcohol
* β- mercaptoethanol

Important note

DNase Stop Buffer: Add the alcohol as the volume marked on bottle label and mix well.

Wash Buffer: Add the alcohol as the volume marked on bottle label and mix well.

Procedure

1. Add 175μl RLysis Buffer and ≤30mg sample into a 1.5ml or 2.0ml centrifuge tube, mix well. For liquid sample, it's 75μl RLysis Buffer and 100μl sample.
2. Add 350μl RD Buffer, mix well. Incubate in 70℃ for 4min. Please overtime for hard sample.
3. Centrifuge at 12,000rpm for 10min,and transfer the supernate into a new tube.
4. Add 200μl absolute alcohol, mix well. Transfer the mixture into a Spin column and centrifuge at 12,000rpm for 60 seconds.
5. Add 600μl Wash Buffer into the Spin column, centrifuge at 12,000rpm for 30 seconds and discard the flow-through.
6. Add the mixture of 40μl DNase Buffer, 9μl $MnCl_2$ and 1μl DNase I into the Spin Column. Incubate in room temperature for 15min.
7. Add 200μl DNase Stop Buffer into the Spin column, centrifuge at 12,000rpm for 30 seconds and discard the flow-through.
8. Add 600μl Wash Buffer into the Spin column, centrifuge at 12,000rpm for 30 seconds and discard the flow-through.
9. Add 250μl Wash Buffer into the Spin column, centrifuge at 12,000rpm for 60~120 seconds and discard the flow-through. And transfer the Spin column to a new 1.5ml centrifuge tube (RNase-free).
10. Add 50~100μl RElution Buffer（or RNase-free water pH>7.0）to the central of the membrane; Incubate at the room temperature for 1 minute.
11. Centrifuge at 12,000rpm for 60 seconds. Remove the Spin column and discard. Then the buffer in the centrifuge tube contains the total RNA.
12. The RNA can be applied kinds of tests. Store the RNA at -80℃ if not be used immediately.

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Troubleshooting

1. We ensure the stability of the Kit should be 18 months in the correct method.
2. Centrifuge at 12,000rpm—14,000rpm at room temperature exception for note.（if possible at 4℃）.
3. RNA may be impaired by the types of oxidation through the extraction, and the oxidation leads to low yield or yield unsteadiness, it is more obviously in oxidase content rich samples. Adding β-Mercaptoethanol into the RLysis Buffer will suppress the impairment effectively. The ratio is 20μl β-ME/ml RLysis Buffer. After adding β-ME, the RLysis Buffer should be stored at 4℃.
4. DNase I is reacting in the menbrane, avoiding RNase contaminant exactly. The mixture of DNase Buffer, $MnCl_2$ and DNase I should not be incubated in low temperature after being mixed to avoid the insufficient digestion which because of low temperature.
5. Add the alcohol (as the volume marked on bottle label) to the DNase Stop Buffer and mix them well.
6. Add the alcohol (as the volume marked on bottle label) to the Wash Buffer and mix them well.
7. Please wear gloves and clean all tips and Eppendorf tubes with DEPC-ddH_2O in order to avoid RNase contaminant. If conditions permit, please operate in ultra-clean cabinet.
8. The RNA should be stored at 4℃ or in the ice bath in order to be used in the downstream experiment, e.g. RT-PCR; or be stored at -80℃. It is suggest avoiding long time storage because RNA is extremely easy to be degraded even with any methods. It is suggested that to store RNA sample in nitrogen or stock buffer in order to ensure purity of RNA.

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Appendix

RNA purification evaluation

RNA yield is determined by measuring the concentration of RNA in the eluate by its absorbance at 260 nm. Absorbance readings at 260 nm should fall between 0.1 and1.0 to be accurate.

Two formulations involved in DNA quantification is shown below:

Concentration of RNA sample = 40 μg/ml x *OD*260 x dilution factor

Ratio= (OD260-OD320)/(OD280-OD320)

For accurate values, we recommend measuring absorbance in 10 mM Tris-HCl, pH 7.5.Inhibitor and ion contaminant can be evaluated by the following RT-PCR, Nouthern or other experiments.

Analysis RNA

⊕ **Absorbance anlysis**

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

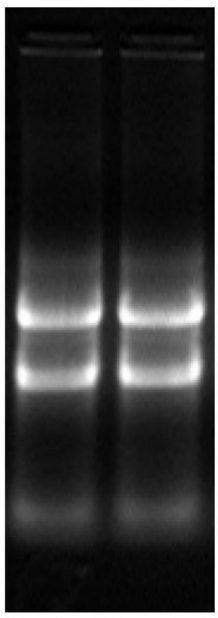
Survey the OD_{260} ， OD_{280} and OD_{320}.

expressions：concentration（μg/ml）＝40×OD_{260}×dilution fact

Target：$2.0 \geq OD_{260-320} / OD_{280-320} \geq 1.7$

Notice: $1.0 \geq OD_{260} \geq 0.1$, the result of ratio is much reliable.

⊕ **Agarose Gel Analysis**



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