常见问题及对策

1. 试剂盒（除DNase I外）室温保存，买到试剂盒后，请及时将DNase I保存于-20 ℃，保质期18个月。
2. 离心速度除已说明外均为12,000rpm，室温离心（有条件可4℃离心）。
3. RNA 在提取过程中可能会受到各种氧化作用的干扰，从而导致 RNA 提取得率下降或不稳定，特别是在氧化酶含量丰富的样本中尤为明显。在 RLysis Buffer 中添加 β-巯基乙醇可有效抑制这些干扰，添加比例为 20μl β-Me/ml Rlysis Buffer，加入 β-巯基乙醇后的 RLysis Buffer 应保存于 4℃。
4. DNase I 反应是在结合膜上进行，应特特别注意避免 RNA 酶的污染。DNase Buffer、 $MnCl_2$ 与 DNase I 混合后不用低温再次保存，以免使用时温度过低而造成消化不充分。
5. 首次使用时在DNase Stop Buffer 和Wash Buffer 瓶中加入标签标明量的无污染的无水乙醇，拧紧瓶盖，并混合均匀。
6. 在使用前请先按照瓶身标签标明体积加入无水乙醇，并将其混匀。
7. 避免环境中RNA 酶污染，操作要戴手套，所有枪头和eppendorf 管以DEPC 水处理，移液枪保证洁净无RNA 酶污染。如果把试剂放在超净台（普通超净台或专用的桌面PCR 超净台）中操作，可以有效减少RNA 酶污染。
8. 提取的总RNA 置于4℃或冰浴中，立即用于下游实验（如RT-PCR）；或立即置于-80℃冰箱保存。无论采用何种方法提取和保存的RNA，都往往会被很快降解，因而建议避免提取RNA 后保存。建议先将标本保存在RNA 保存液或液氮中，来保证RNA 质量不受影响，用试剂盒抽提仍可以获得高质量的RNA。

Biospin Total RNA Extraction Kit

Biospin 总 RNA 提取试剂盒

Cat# BSC63S1

TECHNICAL SUPPORT:

For technical support, please dial phone number ： 0086-571-87774567-5278 or 5211, or fax to 0086-571-87774303
email to reagent@bioer.com.cn.
Website: www.bioer.com.cn

仅供研究、不用于临床诊断

Kit Components (50T)

Component	Amount
RLysis Buffer	8.75ml
RD Buffer	17.5ml
DNase Buffer	2ml
$MnCl_2$	450ul
DNase I	50ul(stored at -20℃)
DNase Stop Buffer	5 ml (add 6.5 ml ethanol before use)
Wash Buffer	32 ml(add 48ml ethanol before use)
RElution Buffer	10ml
Spin columns	50
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.

需要的配套设备和材料

＊ 无菌无酶的1.5ml离心管
＊ 离心机（最大转速＞14,000rpm）
＊ 漩涡振荡器
＊ 无菌无酶的各种规格移液器吸头
＊ 无酶无水乙醇
＊β-巯基乙醇

重要提示

DNase Stop Buffer在使用前请按照瓶身标签标明体积加入无水乙醇，并将其混匀。

Wash Buffer 在使用前请按照瓶身标签标明体积加入无水乙醇，并将其混匀。

操作步骤

1. 取 175μl RLysis Buffer 加入 1.5 或 2.0ml 微量离心管中，加入不多于 30mg 经液氮研磨的样本，并振荡混合均匀。对于液体状态样本，请加入 75μl RLysis Buffer 与 100μl 样本。
2. 加入 350μl 的 RD Buffer，混合均匀后，于 70℃环境中温浴 4 分钟。对难于裂解的样本请适当延长温浴时间。
3. 于 12,000 rpm 离心 10 分钟，吸取上清液至新离心管中。
4. 加入 200μl 无水乙醇，并混合均匀。将混合液体转移至 Spin column。于 12,000 rpm 离心 1 分钟，并弃去接液管中液体。
5. 向 Spin column 中加入 600μl 的 Wash Buffer，于 12,000 rpm 离心 1 分钟，并弃去接液管中液体。
6. 将预先混合的 40μl DNase Buffer、9μl $MnCl_2$ 和 1μl DNase I 加入 Spin column 中，室温放置 15 分钟
7. 向 Spin column 中加入 200μl 的 DNase Stop Buffer 。于 12,000 rpm 离心 30 秒，并弃去接液管中液体。
8. 向 Spin column 中加入 600μl 的 Wash Buffer，于 12,000 rpm 离心 30 秒，并弃去接液管中液体。
9. 向 Spin column 中加入 250μl 的 Wash Buffer，于 12,000 rpm 离心 60~120 秒，并转移至一个新的 1.5ml 离心管（RNase free）。
10. 向 Spin column 中加入 50~100μl RElution Buffer，并于室温温育 1 分钟。
11. 于 12,000 rpm 离心 1 分钟，并弃去 Spin column。1.5ml 离心管中液体含有 RNA。
12. 提取的 RNA 可直接用于各种下游应用实验，如不立即使用，请于-80℃保存。

试剂盒组成（50T）

组分	数量
RLysis Buffer	8.75ml
RD Buffer	17.5ml
DNase Buffer	2ml
$MnCl_2$	450ul
DNase I	50ul (保存于-20℃)
DNase Stop Buffer	5 ml (使用前加入 6.5 ml 无水乙醇)
Wash Buffer	32 ml (使用前加入 48 ml 无水乙醇)
RElution Buffer	10ml
Spin columns	50
说明书	1 份

储存与运输

- 该提取试剂盒于室温保存，DNase I 保存于-20℃，有效期为 18 个月。
- 试剂盒可在常温下运输。

介绍

本试剂盒可直接从各类样本中，如动物组织、细胞、细菌等（不推荐植物组织）提取总 RNA。首先使用 RLysis Buffer 处理样本，RD Buffer 去除蛋白，DNase I 去除 DNA，加入乙醇后使得 RNA 结合于 Spin column，再经过洗涤、洗脱操作即可得到 RNA。

本试剂盒操作简便易行,可以同时处理多个样品,得到高质量的总 RNA。纯化的 RNA 可以直接用于 RNA 印迹分析、斑点杂交、体外翻译、RNA 酶保护分析、RT-PCR 分析、构建 cDNA 文库等 RNA 研究。

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.

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.

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≥$OD_{260-320}$/ $OD_{280-320}$≥1.7

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

⊕ Agarose Gel Analysis