3. 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。

4. 向 Spin column 中加入 250μl 的 Wash Buffer，于 12,000 rpm 离心 2 分钟，并弃去接液管，将 Spin Column 转移至一个新的 1.5ml 离心管。

5. 向 Spin Column 中加入 50μl RElution Buffer，并于室温温育 1 分钟。

6. 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。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。

常见问题及对策

1. 试剂盒室温保存，保质期24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. 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。
5. 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。
6. 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。
7. 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。
8. 抽提仍可以获得高质量的RNA。

Biospin Virus RNA Extraction Kit

Biospin 病毒 RNA 提取试剂盒

Cat# BSC62S1

TECHNICAL SUPPORT:

For technical support, please dial phone number ： 0086-571-87774567-5216 or 5211, 800-857-1279 or fax to 0086-571-87774553
email to reagent@bioer.com.cn.
Website: www.bioer.com.cn

Kit Components (50T)

Component	Amount
RLysis Buffer	15ml
RD Buffer	17.5ml
DNase Stop Buffer	5.0ml(add 6.5ml ethanol before use)
Wash Buffer	20ml(add 30ml ethanol before use)
RElution Buffer	10ml
Spin Columns	50
Handbook	1copy

Storage and transportation

- The Kit has demonstrated stability of 24 months when stored at room temperature.
- The kit can be transported at room temperature.

Introduction

The kit is a ready-to-use reagent for the isolation of Virus RNA from different types sample. Add RLysis Buffer to the processed sample and adding alcohol will bind RNA to spin column. 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.

Technical Information

Sample	Amount
Animal tissue	≤30mg
Culture cells	≤1×10^{8}
White blood cells	≤from 5ml whole blood
For liquid sample	≤100μl

重要提示

RD Buffer 在环境温度低时可能形成混浊，可在37℃水浴加热几分钟，即可恢复澄清。

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

一、 样本前处理

1） 针对动物组织(≤30mg)、培养细胞(≤1×10^{8})、血液白细胞(≤5ml 全血)

1.a **动物组织：**将样本在液氮或冰浴环境中充分研磨，取≤30mg 至预加了 175μl RLysis Buffer 1.5ml 离心管，并混合均匀。

1.b **培养细胞：** (用胰酶消化) 1,500rpm 离心 5min，弃上清，加入 175μl Rlysis Buffer 并混合均匀。

1.c **血液白细胞：**使用**红细胞裂解液**（Cat#BSA06M1）处理全血获得白细胞，加入 175μl RLysis Buffer 并混合均匀。

2. 加入 350μl RD Buffer 混合均匀于 70℃温浴 4min。

3. 于 12,000 rpm 离心 10 分钟，吸取上清液至新离心管中。加入 200μl 无水乙醇，并混合均匀。

2） 针对细胞(≤1×10^{3})、血液白细胞(≤0.3ml 全血)、全血、血浆、血清、腹水等

1.a **细胞：** (用胰酶消化) 1,500rpm 离心 5min，弃上清。用 100μl PBS 重悬，加入 300μl RLysis Buffer 并混合均匀。

1.b **血液白细胞：**使用**红细胞裂解液**（Cat#BSA06M1）处理全血获得白细胞，加入 300μl RLysis Buffer 并混合均匀。

1.c **全血、血浆、血清、腹水等液体样本：**取 100μl (不足 100μl 请用 PBS 补足到 100μl)，加入 300μl RLysis Buffer 并混合均匀。

2. 加入 400μl 无水乙醇，并振荡混合均匀。

二、 样本提取操作

1. 取上述混合液（**≤750**μl）转移至 Spin Column，于 10,000 rpm 离心 1 分钟，并弃去接液管中液体。

2. 向 Spin column 中加入 200μl DNase Stop Buffer，于 12,000 rpm 离心 30 秒，并弃去接液管中液体。

试剂盒组成（50T）

Component	Amount
RLysis Buffer	15ml
RD Buffer	17.5ml
DNase Stop Buffer	5.0ml(使用前加入 6.5ml 乙醇)
Wash Buffer	20ml(使用前加入 30ml 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，有效期为 24 个月。
- ◆ 可在常温下运输。

介绍

本试剂盒是一种从各种样本提取病毒 RNA 的提取试剂盒,可直接从各种样本的病毒提取 RNA。首先使用 RLysis Buffer 处理样本，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 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。

基本技术参数

样本	Amount
动物组织	≤30mg
培养细胞	≤1×10^8
血液白细胞	≤from 5ml whole blood
液体样本	≤100µl

需要的配套设备和材料

* 无菌无酶的1.5ml离心管和移液器吸头
* 无酶无水乙醇和PBS
* 可选择是否使用β-巯基乙醇
* 70℃水浴装置
* 离心机（最大转速＞12,000rpm）
* 漩涡振荡器和70℃加热装置
*红细胞裂解液（Cat#BSA06M1）
*液化氮（或冰块）

Apparatus and materials to be prepared by the user

* Sterile 1.5ml microcentrifuge tubes
* 10µl/100µl/1000µl tips
* microcentrifuge capable of 14,000rpm
* 70℃ water bath
* Vortex mixer
* Liquid nitrogen (or ice bath)
* optional: β-Me
* PBS
*Trypsination
*Red Blood Cells Lysis Buffer（Cat#BSA06M1）
* Absolute alcohol

Important note

RD Buffer may be precipitated at low temperature, the heated at 37 ℃ for a few minutes, to restore the clarification.

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

Ⅰ Sample pretreatment

1) For animal tissue(≤30mg), culture cells(≤1×10^8), white blood cells(≤from 5ml whole blood)

1.a **Animal tissue:** Homogenize the tissue under liquid nitrogen or ice bath, and transfer up to 30mg to a 1.5ml tube which was added 175µl RLysis Buffer. Mix well.

1.b **Culture cells:** (Detach cells by trypsination), then centrifuge at 1,500rpm for 5min, discard the supernatant. Add 175µl RLysis Buffer and mix well.

1.c **White blood cells:** Get white blood cells form whole blood by using **Red Blood Lysis Buffer** (Cat# BSA06M1). Add 175µl RLysis Buffer and mix well.

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. Add 200µl alcohol, mix well.

2) For culture cells(≤1×10^3), white blood cells(≤from 0.3ml whole blood), whole blood, plasma, serum, ascites and so on.

1.a **Culture cells:** (Detach cells by trypsination), then centrifuge at 1,500rpm for 5min, discard the supernatant. Add 300µl RLysis Buffer and mix well.

1.b **White blood cells:** Get white blood cells form whole blood by using **Red Blood Lysis**

Buffer (Cat# BSA06M1). Add 300µl RLysis Buffer and mix well.

1.c **Whole blood, plasma, serum, ascites and so on:** Transfer 100µl sample(If the sample volumn ≤100µl, add PBS to 100µl), and add 300µl RLysis Buffer. Mix well.

2. Add 400µl alcohol and mix well.

Ⅱ Sample extraction operation

1. Transfer the supernate into a Spin Column and centrifuge at 10,000rpm for 60 seconds.
2. Add 200µl DNase Stop Buffer into the Spin Column, centrifuge at 12,000rpm for 30 seconds and discard the flow-through.
3. Add 600µl Wash Buffer into the Spin Column, centrifuge at 12,000rpm for 30 seconds and discard the flow-through.
4. Add 250µl Wash Buffer into the Spin Column, centrifuge at 12,000rpm for 120 seconds and discard the flow-through.
5. Transfer the Spin Column to a new1.5ml microcentrifuge tube.
6. Add 50µl RElution Buffer（or RNase-free water pH>7.0）to the central of the membrane; Incubate at the room temperature for 1 minute.
7. Centrifuge for 1 minute. Remove the Spin Basket and discard. Then the buffer in the microcentrifuge tube contains the RNA.

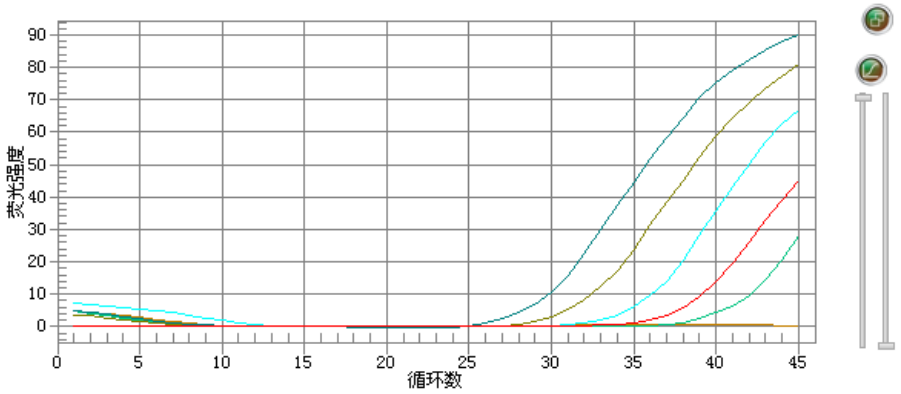
The RNA can be applied kinds of tests. Store the RNA at -80℃ if not be used immediately.

Troubleshooting

1. We ensure the stability of the Kit should be 24 months in the correct method.
2. Centrifuge at 12,000rpm 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. Add the alcohol (as the volume marked on bottle label) to the DNase Stop Buffer and mix them well.
5. Add the alcohol (as the volume marked on bottle label) to the Wash Buffer and mix them well.
6. 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.
7. 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.

Analysis RNA

Example: HCV Real-time RT-PCR （Sample HCV concentration：copies/ml）



Sample	1E07	1E06	1E05	1E04	1E03	NC
Ct	25.83	28.88	32.36	35.87	38.73	--