

User Manual

Version 3.0

总 RNA 快速提取试剂盒

Easy RNA Extraction Kit

Catalog Number DR0401050



EASY-DO

易思得生物



总 RNA 快速提取试剂盒

产品简介

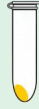
本试剂盒可从一定量的生物样品（非多糖多酚类）中快速提取高纯度 RNA。本试剂盒操作简单，仅需~10 分钟即可完成提取工作，RNA 提取得率高，所得 RNA 可以直接用于 Northern 杂交、mRNA 纯化、体外翻译、RT-PCR 等 RNA 相关的分子生物学实验。

产品组成

试剂盒组成	50 次 (DR0401050)
Buffer UR1	50 ml
Buffer UR2	15 ml
Buffer CR（氯仿替代物）	10 ml
RNA Elution Buffer	05 ml
HiPure RNA Column	50 个
2 ml Collection Tube	50 个
1.5 ml Collection Tube	50 个
说明书	1 份

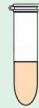
操作流程示意图

取样



取 $5 \times 10^5 \sim 1 \times 10^7$ 细胞或 5~30 mg 组织

处理



先加 1 ml Buffer UR1 反复吹打，再加 200 μ l 氯仿（需自备）或 Buffer CR 剧烈混匀 15 s

吸附



离心后取上清 300 μ l，
加 300 μ l 异丙醇或无水乙醇混匀后上柱

漂洗



加 600 μ l Buffer UR2 漂洗（重复一次）

洗脱



加 20~30 μ L RNA Elution Buffer

不同来源样品的处理

培养的动物细胞:

贴壁细胞: 每 10 cm² 的培养皿中加入 **1ml Buffer UR1** 试剂（如直径为 3.5 cm 细胞培养皿，吸弃培养基后，往培养皿中加入 **1ml Buffer UR1**），用移液器反复吹打使细胞充分裂解，将裂解液转移到 2 ml 无 RNA 酶的离心管中，进入步骤 3 的操作。

悬浮细胞: 将含悬浮细胞的培养液转移至离心管中，8,000g，4℃离心 2 分钟，弃上清，注意不要破坏细胞沉淀，收集约 $5 \times 10^5 \sim 1 \times 10^7$ 细胞，加入 **1ml Buffer UR1** 试剂，用移液器反复吹打使细胞充分裂解，将裂解液转移到 2 ml 无 RNA 酶的离心管中，进入步骤 3 的操作。

普通动植物组织:

在研钵中用液氮将样品研磨至粉末状，再用液氮预冷的 2 ml 无 RNA 酶的离心管称取约 5~30 mg 研磨成粉末状的组织，加入 **1ml Buffer UR1** 试剂，用移液器反复吹打使细胞充分裂解，进入步骤 3 的操作。

操作步骤

- 1. 取样:** 将 $5 \times 10^5 \sim 1 \times 10^7$ 的细胞或者经液氮研磨成粉末的 **5~30 mg 动植物组织** 收集至 2 ml 无 RNA 酶的离心管（自备）中。
- 2. 裂解:** 往离心管内加入 **1ml Buffer UR1**，用移液器吹打或涡旋 **15 s**，使细胞充分裂解。
- 3. 释放:** 往离心管内加入 **200 μ l 氯仿（需自备）或 Buffer CR**，剧烈混匀 **15 s**，最高速（ $\geq 13,000$ g）室温离心 3-5min。
- 4. 上柱:** 小心吸取上清液 **300 μ l**，加入**等体积的异丙醇或者无水乙醇**，颠倒混匀后将溶液转移至套放于收集管的 HiPure RNA 吸附柱中，12,000 rpm（ $\geq 13,000$ g）室温离心 30 s。
- 5. 洗涤:** 将吸附柱放回收集管中，加入 **600 μ l Buffer UR2**，12,000 rpm（ $\geq 13,000$ g）室温离心 30 s，弃废液。重复洗涤一次。
注：确认已往 Buffer UR2 中加入指定体积（见瓶身）的无水乙醇。
- 6. 挥发:** 吸附柱放回收集管中，12,000 rpm（ $\geq 13,000$ g）室温离心 1 min。
- 7. 洗脱:** 将吸附柱放入洁净的 1.5 ml 无 RNA 酶的离心管中，在膜中央加入 **20~30 μ l RNA Elution Buffer***，室温放置 1~2 min，12,000 rpm（ $\geq 13,000$ g）室温离心 1 min，洗脱液即含 RNA。
***将 RNA Elution Buffer 加热至 65°C，可提高洗脱效率。**
- 8. 检测:** 取 1~5 μ l 进行检测，洗脱液即可用于后续实验或 -80°C 冻存。

保存条件

本试剂盒 **Buffer UR1**、**Buffer CR** 需于 4°C 避光保存，其余组分于室温保存；所有瓶盖须旋紧，避免试剂失效；**Buffer UR2 在使用前须加入指定体积（见瓶身）的无水乙醇**；**Buffer UR1** 中含有机试剂，请在操作时戴好口罩和手套，若不慎接触到皮肤或眼睛，请立即用大量清水冲洗并求医。

产品问答

Q1. 细胞 RNA 的提取得率为何偏低？

A1. 可从以下几个方面考虑：

- （1）实验细胞量太少，细胞量建议不少于 5×10^5 。
- （2）细胞量太多，裂解不充分，RNA 未充分释放。请降低细胞量或根据细胞量进行分管操作。
- （3）若实验样本为多糖多酚类植物样本，多糖类物质和 Buffer UR1 形成不可溶解的沉淀，严重影响 RNA 的释放。

Q2. 提取的 RNA 为何会有降解？

A2. RNA 的降解主要由以下几个环节导致：

- （1）使用的生物材料不够新鲜。应采用新鲜的生物材料，或将新鲜的生物材料用液氮迅速冷冻后置于 -80°C 保存。
- （2）起始细胞或组织量过大导致裂解不充分，请按试剂盒推荐比例进行操作。

产品问答

Q3. 实验细胞量若超出说明书规定用量时怎么办？

A3. 当实验细胞量超出说明书的用量范围时，可根据推荐比例，增大试剂用量或分成多管进行操作。

Easy RNA Extraction Kit

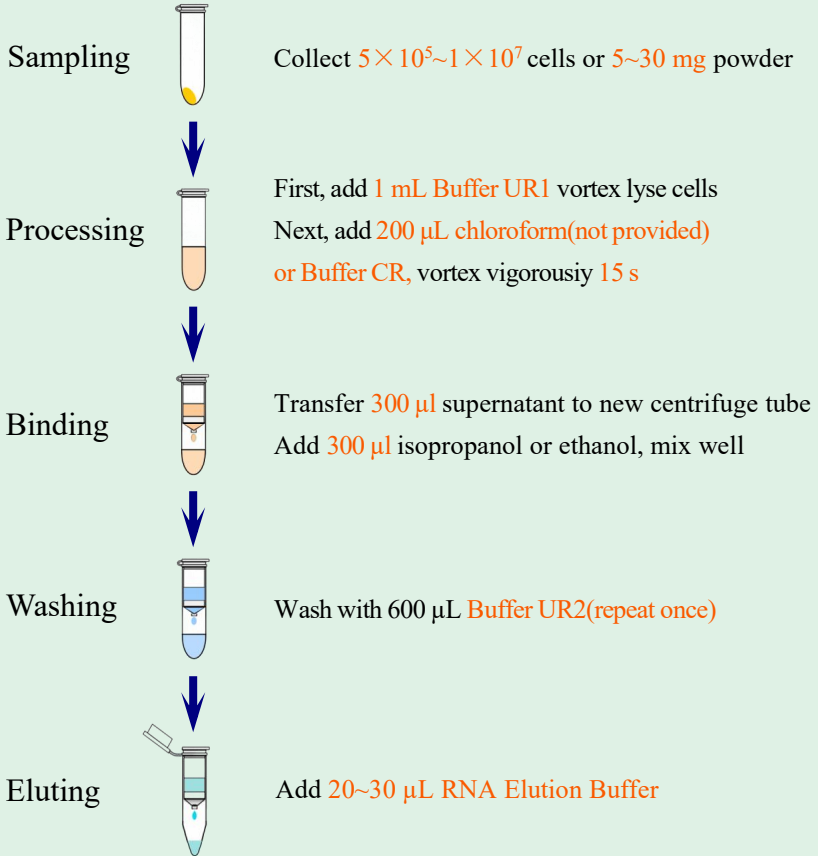
Product introduction

This kit can isolate total RNA easily and rapidly from animal or plant tissues (non-polysaccharides and non-polyphenols) and cultured cells. The kit is easy to operate, and it takes only 10 minutes to complete the extraction process. The isolated total RNA is intact with little DNA or proteins, thus it can be used for RNA related assays such as RT-PCR, Northern blot analysis, mRNA isolation, and *in vitro* translation reactions.

Kit Contents

Cat. No.	50 reactions (DR0401050)
Buffer UR1	50 ml
Buffer UR2	15 ml
Buffer CR	10 ml
RNA Elution Buffer	05 ml
HiPure RNA Column	50
2 ml Collection Tube	50
1.5 ml Collection Tube	50
Handbook	1

Experimental Overview



Treatments of different samples

Cultured cells

Adherent cells: Add 1ml Buffer UR1 onto a 10 cm² petri dish with adherent cells, swirl the reagent around in plate to make sure the dish has been covered with Buffer UR1 (1 ml Buffer UR1 was added in cell culture dish with diameter of 3.5 cm). Repeatedly pipette several times until cells are completely resuspended. Collect the cells with a pipette and transfer them to the 2 ml RNase-free centrifuge tube and the operation proceeds to step 3.

Non-adherent cells: Transfer the cells and media into a centrifuge tube, centrifuge at 8,000 g for 2 minutes at 4 °C, discard the supernatant and avoid disturbing the cell pellet. Add 1 ml Buffer UR1 for every $5 \times 10^5 \sim 1 \times 10^7$ cells. Pipette up and down until cells are fully lysed, and the lysate was transferred to a 2 ml RNase-free centrifuge tube and the operation proceeds to step 3.

Animal and plant tissue sample

The sample was ground to a powder in liquid nitrogen using a mortar and pestle. About 5~30 mg of the tissue powder was weighed in a 2 ml RNase-free centrifuge tube which was precooled with liquid nitrogen, and 1 ml of Buffer UR1 was added, then pipette up and down until cells are fully lysed and the operation proceeds to step 3.

Procedure

1. **Sampling:** Immediately transfer frozen or fresh tissue into mortar, add liquid nitrogen, then crush with pestle to homogenize until powdery. Transfer the tissue powder (about **5~30 mg** powder) into 2 ml RNase-free centrifuge tube.
2. **Lysing:** Add **1 ml Buffer UR1** and then pipette up and down or vortex 15s until cells are fully lysed.
3. **Reacting:** Add **200 μ l chloroform(not provided) or Buffer CR**, vortex and mix 15s, and then centrifuge at maximum speed ($\geq 13,000$ g) for 3-5min.
4. **Binding:** Transfer **300 μ l** top liquid layer to new centrifuge tube without touching middle layer. Add **300 μ l** of **isopropanol or ethanol** for homogenization and mix well. Place a HiPure RNA Column in a provided 2 ml collection tube. To bind RNA, apply **600 μ l mixture** to the HiPure RNA Column, and then centrifuge at 12,000 rpm ($\geq 13,000$ g) for 30 s. Discard flow-through and place HiPure RNA column back into the same collection tube.
5. **Washing:** Add **600 μ l Buffer UR2** to HiPure RNA Column and 12,000 rpm ($\geq 13,000$ g) centrifuge for 30 s. Discard flow-through and place HiPure RNA column back into the same collection tube. Repeat the washing procedure again.

For Research Only

Note: Make sure that 96–100% ethanol has been added to Buffer UR2.

6. **Volatilizing:** Discard the flow-through and centrifuge the HiPure RNA Column for 1 min at 12,000 rpm ($\geq 13,000$ g).
7. **Eluting:** Place HiPure RNA Column into an RNase-free 1.5 ml centrifuge tube. Add **20~30 μ l RNA Elution Buffer***, leave it at room temperature for 1-2 min, centrifuge at 12,000 rpm ($\geq 13,000$ g) for 1 min at room temperature. The liquid in the tube is the solution containing the target RNA.

Note: Heating RNA Elution buffer to 65°C improves elution efficiency.

8. **Testing:** The purified RNA can be checked by agarose gel analysis. The obtained RNA can be used for follow-up experiments immediately or be stored at -80°C.

Storage

The **Buffer UR1 and Buffer CR** should be stored dry at 4 °C while other contents are stored at room temperature, and all caps must be tightly closed to avoid reagent failure. **Add absolute ethanol to Buffer UR2 before use (see bottle label for the detailed volume)**. If the skin or eyes accidentally touch the reagent, especially the **Buffer UR1** which containing organic reagents, rinse it immediately with plenty of water and see a doctor.

Note

All centrifugation steps are performed at room temperature since the addition of Buffer UR1.

Q&A

Q1. Why the extraction rate of RNA is low?

A1. When the RNA extraction rate is low, it can be considered from the following aspects:

- ① The initial sample amount is too little to effectively isolate the RNA, please increase the sample size.
- ② If the initial sample volume is too large, the Buffer UR1 might be not sufficient. Please operate the assay according to the recommended ratio of the kit.
- ③ The polysaccharides and non-polyphenols which present in some plant tissues would form insoluble precipitation with Buffer UR1, causing low extraction rate of RNA.

Q2. Why does the extracted RNA degraded?

A2. When the extracted RNA is degraded, it can be considered from the following aspects:

- ① Tissue used for RNA extraction was not fresh. It is strongly recommended that the samples should be fresh or flash frozen with liquid nitrogen then stored at -80°C .
- ② Too large amount of tissue was used. Add the suggested ratio of Buffer UR1 to the samples.

Q&A

- Q3.** What should I do if the sample size exceeds the amount specified in the manual?
- A3.** This kit is mainly used for small-scale preparation of RNA. When the sample size exceeds the range of the specification, it can be operated through increasing the reagent dosage according to the recommended ratio or dividing into multiple tubes. Too large initial amount will affect the lysis efficiency, resulting in low yield or even experimental failure.



易思得
Easy-Do, make it easier!

浙江易思得生物科技有限公司

Zhejiang Easy-Do Biotech CO.,LTD

服务热线：0571-87246065

公司网站：www.zj-easydo.com