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	$\leqslant 1\times 10^{8}$
White blood cells	⩽from 5ml whole blood
For liquid sample μl	⩽100

Apparatus and materials to be prepared by the user

* Sterile 1.5ml microcentrifuge tubes * 10μl/100μl/1000μl tips
* microcentrifuge capable of 14,000rpm * 70℃ waterbath *Vortexmixer
* 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 350 μl 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 300 μl 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 400 μl 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 200 μl DNase Stop Buffer into the Spin Column, centrifuge at 12,000rpm for 30 seconds and discard the flow-through.
3. Add 600 μl Wash Buffer into the Spin Column, centrifuge at 12,000rpm for 30 seconds and discard the flow-through.
4. Add 250 μl 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 50 μl 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.

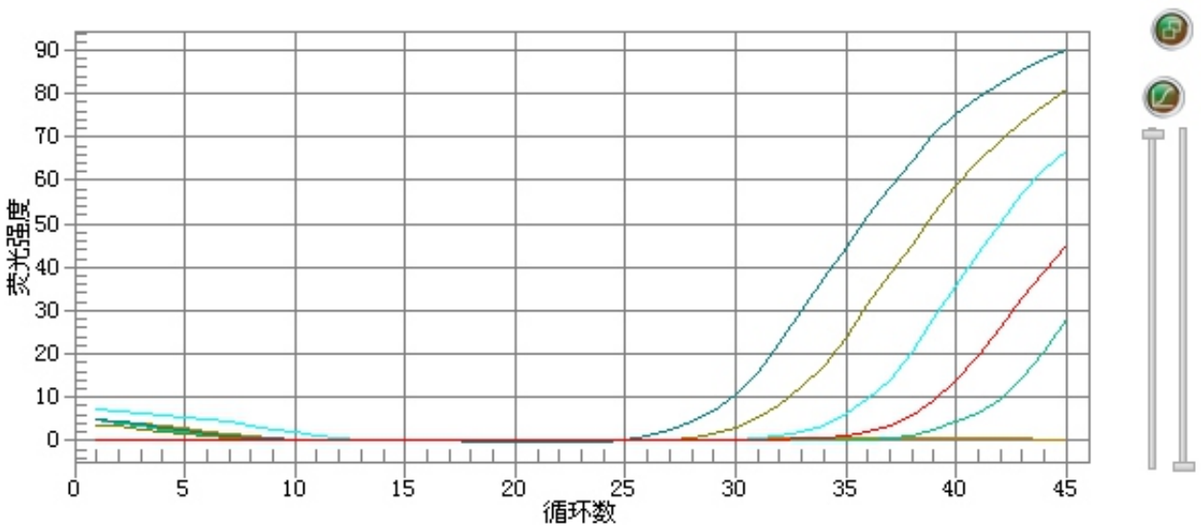
The RNA can be applied kinds of tests. Store the RNA at -80℃ if not be used immediately.

Troubleshooting

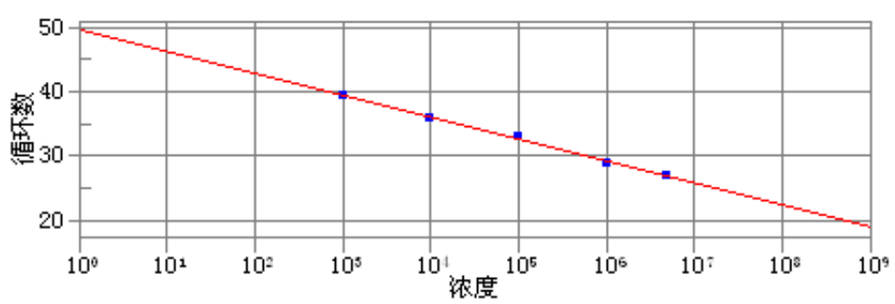
1. We ensure the stability of the Kit should be 24 months in the correct method.
2. Centrifuge at 12,000rpm at room temperature exception for note.（if possible at 4℃）.
3. RNA may be impaired by the types of oxidation through the extraction, and the oxidation leads to low yield or yield unsteadiness, 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	--



Y轴截距	49.48
斜率	-3.38
误差	0.008
相关系数	-0.999