HiYield RNA UltraPurification Kit

Cat. No.:	YUR100	YUR300
Product Name:	HiYield RNA UltraPurification Kit	
Reactions:	100	300
RNA Size Range:	200nt-10knt	
Recovery:	Up to 95%	
Format:	Spin Column	
Operation:	Centrifuge	
Operation Time:	Within 20 Minutes	

Introduction

HiYield RNA UltraPurification Kit is especially designed to recover or concentrate RNA fragments (200nt-10knt) from all enzymatic reactions. RNA fragments previously isolated with RBC kits in reagent format or with other isolation methods could be fast and easily concentrated with this kit. The unique UR Buffer and high yield UR column make this kit exceptional value. Salts, enzymes and unincorporated nucleotides can be effectively removed from the reaction mixtures without phenol extraction or alcohol precipitation. The average recovery is around 80-90%. The entire procedure can be completed in 20 minutes.

Features

1. High and reproducible recoveries for constant results.
2. Ready-to use highly concentrated RNA within 20 minutes.
3. Without phenol extraction or alcohol precipitation.

Contents

ITEM	YUR100	YUR300
UR Buffer	80ml	240ml
Wash Buffer*	25ml	50ml
Elution Buffer	6ml	30ml
UR Column	100pcs	300pcs
2ml Collection Tube	100pcs	300pcs

*Absolute ethanol shall be added to the Wash Buffer prior to the initial use. Please refer to the bottle label for details.

Applications

The highly concentrated RNA is suitable for direct use in applications such as: RT-PCR, Northern Blotting, Primer Extension, mRNA Selection and cDNA Synthesis.

Quality Control

The quality of HiYield RNA UltraPurification Kit is tested on a lot-to-lot basis by purifying RNA of various sizes from aqueous solutions. The purified RNA is checked by electrophoresis.

Caution

UR Buffer contains guanidine thiocyanate which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Protocol

Additional Requirements:
Microcentrifuge tubes, absolute ethanol.

Things Before Starting:
1. For YUR100-Add 100ml of absolute ethanol to 25ml of Wash Buffer prior to the initial use. For YUR300-Add 200ml of absolute ethanol to 50ml of Wash Buffer prior to the initial use.
2. Preheat the Elution Buffer to 60℃ prior to the initial use.

Step 1 Sample Preparation	★Transfer up to 100 µl of a RNA product to a 1.5 microcentrifuge tube. ★Add **500ul of UR Buffer** to 100ul of the RNA product and shake vigorously. (The volume of UR Buffer is fivefold the volume of RNA products.)
Step 2 RNA Binding	★Add an equal volume of 70% ethanol (if the sample mixture is 600 µl, add 600 µl of 70% ethanol) to the sample mixture from Step 1 and shake vigorously (break up any precipitate with pipetting). ★Place a **UR Column** in a **2 ml Collection Tube**. ★Transfer the sample mixture from the previous step into the **UR Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and transfer the remaining mixture to the same **UR Column**. ★Centrifuge at full speed for 1 minute. ★Discard the flow-through and place the **UR Column** back in the **2 ml Collection Tube**.
Step 3 Wash	★Add **600 µl of Wash Buffer** (ethanol added) into the center of the **UR Column**. ★Centrifuge at full speed for 30 seconds. ★Discard the flow-through and place the **UR Column** back in the **2 ml Collection Tube**. ★Centrifuge again for 3 minutes at full speed to dry the column matrix.
Step 4 RNA Elution	★Transfer the dried **UR Column** to a new 1.5 ml microcentrifuge tube. ★Add **15-50 µl of Elution Buffer** or TE (RNase-free) into the center of the column matrix. ★Let stand for 2 minutes or until the **Elution Buffer** or TE (RNase-free) is completely absorbed by the matrix. ★Centrifuge for 2 minutes at full speed to elute the purified RNA.

Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	**Incorrect RNA Elution Step** Ensure that the Elution Buffer is completely absorbed after being added to the center of the UR Column.
	Incomplete RNA Elution If the RNA fragments are larger than 10 Kb, use preheated Elution Buffer (60-70ºC) in the Elution Step to improve the elution efficiency.
Eluted RNA does not perform well in downstream applications.	**Residual ethanol contamination** Following the Wash Step, dry the UR Column with additional centrifugation at full speed for 5 minutes or incubate at 60ºC for 5 minutes.
	RNA was denatured (a smaller band appeared on gel analysis) Incubate the eluted RNA at 95ºC for 2 minutes, and then cool down slowly to re-anneal the denatured RNA.