

After Tri-Zol RNA Clean-Up Kit

Description

After Tri-Zol RNA Clean-Up Kit is designed for fast clean up RNA that be isolated by different methods, such as guanidine isothiocyanate/phenol chloroform extraction or lithium chloride / phenol chloroform extraction and is also suitable for fast clean up RNA from enzymatic reaction mixture, such as labeling or DNase digestion reactions. In the purification procedure, combining the efficient reagents with the convenient spin-column system, impurities will remove completely. The entire procedure is not required the phenol-chloroform extraction and can be finished within 10 minutes. After using this purification kit, the purified RNA is ready for RT-PCR and other downstream application.

Features

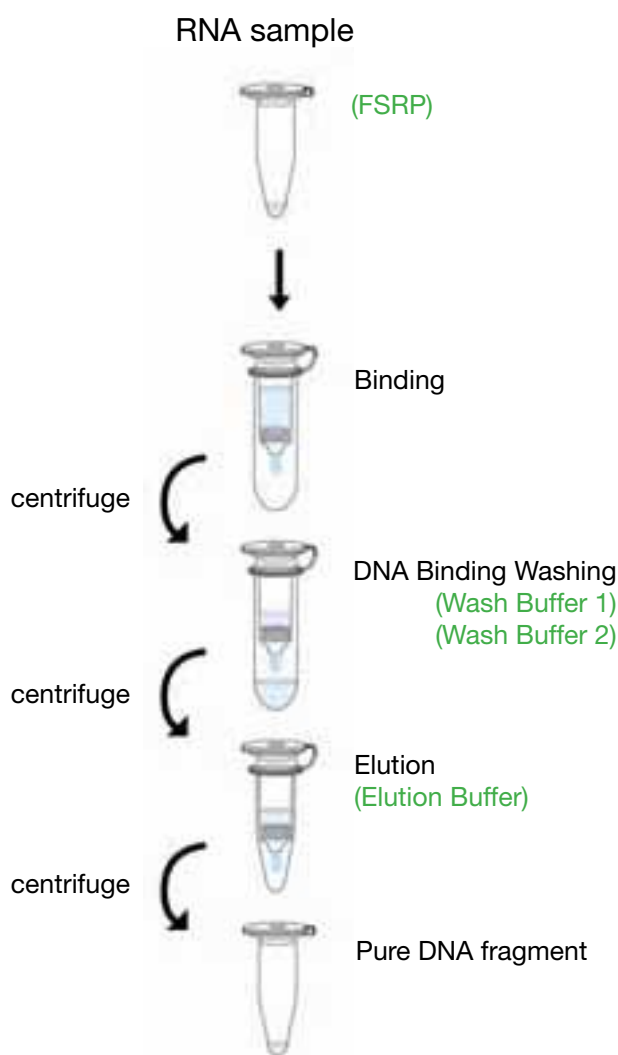
- RNA clean up can be operated directly after the chloroform extraction without isopropanol precipitation.
- **Sample Size:** Up to 100 μ l of RNA sample or enzymatic reaction mixture.
- **High purity:** OD_{260/280}: 1.9~2.1.
- **Binding Capacity:** Up to 100g
- **Handling Time:** Within 10 minutes
- **Expected Recovery:** 85~95%
- **Format:** Spin Column

Applications

- Real-Time PCR
- Northern blotting hybridization
- Primer extension
- Differential display
- RNase protection assays
- As starting material for the purification of mRNA for cDNA synthesis

Storage Conditions

Stable for 1 year at room temperature.



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Ordering Information

Cat. No.	Product Name	Size	Kit Components	Store at
RE-001	RNA Purification Mini Kit (After Tri-Zol)	50 preps.	FSRP Buffer Wash 1 Buffer Wash 2 Buffer RNase-free Water FSRB Mini Column 2.0 ml Collection Tube 1.5ml Elution Tube	Store at room temperature (15~ 25°C) for 1 year.
RE-002	RNA Purification Mini Kit (After Tri-Zol)	200 preps.	FSRP Buffer Wash 1 Buffer Wash 2 Buffer RNase-free Water FSRB Mini Column 2.0 ml Collection Tube 1.5ml Elution Tube	Store at room temperature (15~ 25°C) for 1 year.

Distributed by:



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Kit Contents:

	RE-001 (50 preps)	RE-002 (200 preps)
FSRP Buffer	30 ml	80 ml
Wash 1 Buffer	30 ml	110 ml
Wash 2 Buffer (concentrated)	20 ml	35 ml
RNase-free Water	6 ml	6 ml
FSRB Mini Column	50 pcs	100 pcs
Collection Tube	50 pcs	100 pcs
Elution Tube	50 pcs	100 pcs

*Add 80 ml ethanol (96-100%) to Wash Buffer 2 when first open.

**Add 140 ml ethanol (96-100%) to Wash Buffer 2 when first open.

Specification

Sampl Size : up to 100 µl RNA sample
or enzymatic reaction mixture

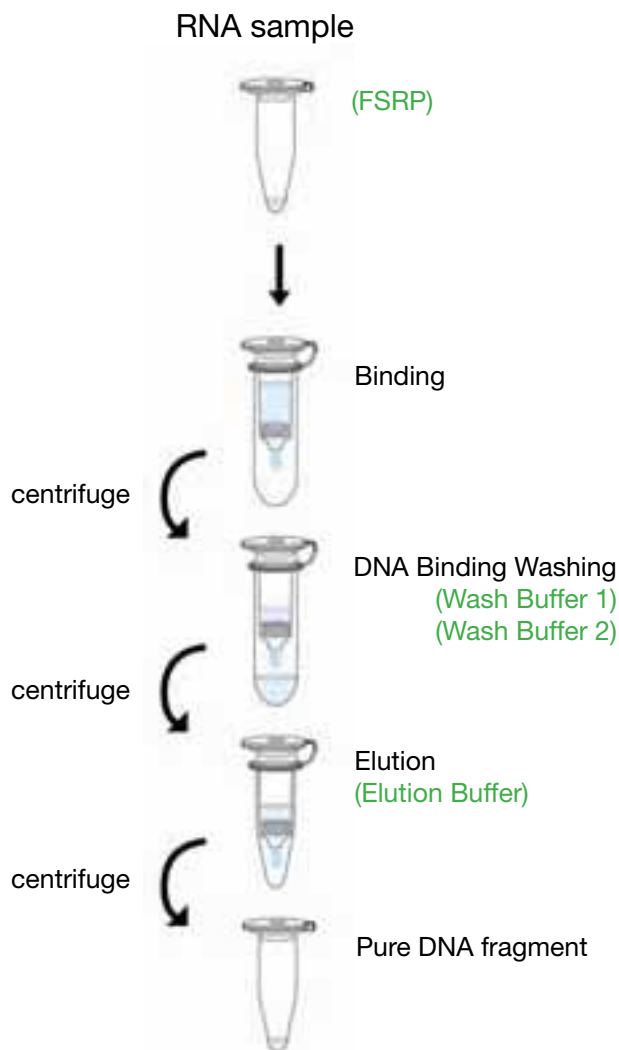
Recovery : 85-95%.

Binding Capacity/ column : up to 100 µg

Handling Time: Within 10 min

Important Notes

1. Make sure everything is RNase-free when handling RNA.
2. Buffer provided in this kit contain irritants.
Wear gloves and lab coat when handling these buffers.
3. Add 6 ml ethanol (96~100%) to Wash Buffer 2 when first open.
4. (For optional step) Dilute RNase-free DNase I in dilution buffer
(150 mM NaCl, 1 mM MgCl₂, 10 mM Tris HCl, pH 7.5)
to final Conc. 0.5U/µl.



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• **General Protocol:**

Please Read Important Notes Before Starting The Following Steps.

1. Adjust the sample volume to 100 μ l with RNase-free water (provided).
 - **The maximum sample volume is 100 μ l.**
2. Add 350 μ l of FSRP Buffer to the sample and vortex vigorously.
3. Add 250 μ l of ethanol (96~100%) to the sample mixture and mix well by vortexing.
4. Transfer the entire ethanol added sample (including any precipitate) to FSRB Mini Column Set. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min and discard the flow-through.
5. (Optional):

To eliminate DNA contamination, follow the steps from 5a. Otherwise, proceed to step 6 directly.

 - 5a. Add 250 μ l of Wash Buffer 1 to wash FSRB Mini Column.
Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.
 - 5b. Add 100 μ l of RNase-free DNase 1 solution (0.5 U/ μ l, not provided) to the membrane center of FSRB Mini Column. Place the Column on the benchtop for 15 min.
 - 5c. Add 250 μ l of Wash Buffer 1 to wash FSRB Mini Column.
Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.
 - 5d. After DNase 1 treatment, proceed to step 7.
6. Add 500 μ l of Wash Buffer 1 to wash FSRB Mini Column. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.
7. Wash FSRB Mini Column twice with 750 μ l of Wash Buffer 2 by centrifuge at full speed (14,000 rpm or 10,000 x g) for 1 min then discard the flow-through.
 - **Make sure that ethanol has been added into Wash Buffer 2 when first open.**
8. Centrifuge at full speed (14,000 rpm or 10,000 x g) for an additional 3 min to dry the column.
 - **Important Step! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.**
9. Place FSRB Mini Column to Elution Tube (provided).
10. Add 30~50 μ l of RNase-free water to the membrane center of FSRB Mini Column. Stand FSRB Mini Column for 1 min.
 - **Important Step! For effective elution, make sure that RNase-free ddH₂O is dispensed on the membrane center and is absorbed completely.**
11. Centrifuge at full speed (14,000 rpm or 10,000 x g) for 2 min to elute RNA.
12. Store RNA at -70C.

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Troubleshooting:

Problem	Possible reasons	Solutions
Little or no RNA eluted	RNA remains on the column	<ul style="list-style-type: none"> • Repeat elution. • Pre-heat DEPC-water to 70° C prior to elution. • Incubate for 5 min with water prior to elution.
Degraded RNA	Source	<ul style="list-style-type: none"> • Follow protocol closely, and work quickly.
	RNase contamination	<ul style="list-style-type: none"> • Ensure not to introduce RNase during the procedure. • Check buffers for RNase contamination.
Problem in downstream applications	Salt carry-over during elution	<ul style="list-style-type: none"> • Ensure Wash Buffer 2 has been diluted with 4 volumes of 100% ethanol as indicated on bottle. • Repeat wash with Wash Buffer 2.
Abnormal OD reading on A260/A280	DEPC residue remains in DEPC-water	<ul style="list-style-type: none"> • Use provided RNase-free water. • Use 10 mM Tris-HCl, not the DEPC water to dilute the sample before measuring purity.

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