

RNA-Zol Direct Clean Up Plus Kit (Trizol included) RE-040 & RE-041

For Cells and Tissues

Description

RNA-Zol Direct Clean up Plus Kit allows RNA purification directly from samples lysed or stored in TRIzol® (or similar). The procedure is fast, easy and allows to perform an optional DNase treatment to remove genomic DNA(gDNA) contaminations prior qPCR analysis. This Kit is indicated for the isolation of **total RNA, including small RNAs (17-200 nt) and miRNA**, from a variety of sample sources such as animal **cells, tissues**, bacteria, yeast, biological fluids.

Highlights

- Spin-column purification of total RNA (including small/microRNAs) directly from TRIzol®, TRI Reagent® or similar acid-guanidinium-phenol based reagents.
- RNA is ready for Next-Gen Sequencing, RT-qPCR, etc. DNase I is included
- **Sample Size:** Up to 500 µl of RNA sample or enzymatic reaction mixture.
- **Binding Capacity:** Up to 100 µg of Total RNA/column
- **Sample Size:** 1x 10⁶ culture cells – up to 20 mg tissues
- **Expected yield:** 10 µg of total RNA per column
- **Format:** Spin Column
- **Minimum elution volume:** 40 µl
- **Handling Time:** Within 30 minutes (20 min if DNase treatment is not performed)

Storage Conditions

Stable for 1 year at room temperature.

Kit components	RE-040S 4 preps	RE-040 100 preps	RE-041 200 preps	Store at
Wash 1 Buffer	2 x 1,5 ml	60ml	2X 60ml	Store at room temp. (15~ 25°C) for 1 year.
Wash 2 Buffer conc.*	2 x 1,5 ml	35 ml *	2X 35 ml *	
RNase-free Water	0,5 ml	6 ml	2X 6 ml	
FSRB Mini Column	4 pieces	100 pieces	2X 100 pieces	
2.0 ml Collection Tube	4 pieces	100 pieces	2X 100 pieces	
1.5ml Elution Tube	4 pieces	100 pieces	2X 100 pieces	
DNase I **	-	1 vial	1X 1 vial	
TriZol reagent	0,5 mL	100 ml	2X 100 ml	

* Add 140 ml ethanol (96%-100%) to Wash Buffer 2 conc. When first open for RE-040 (100 preps)
Add 280 ml ethano to Wash Buffer 2 conc. when first open RE-041 (200 preps)

**All the kits are supplied with DNase I (conc. is 500 U/1 mg)

We suggest to reconstitute 1mg DNase I into 5ml DNase I buffer, to obtain a concentration of 0.1 U/µl *
For RE-040S trizol Is not included

Required Material – not provided: DNase I reaction buffer (1 mM NaCl, 10 mM MgCl₂, 20 mM Tris-HCl, pH 7.0 at 25°C)

*Once reconstituted in its buffer, DNase I solution is stable at + 4°C, we recommend to use it within 6 months

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 ethanol (96~100%) to Wash Buffer 2 (conc) when first open.
4. (For optional step) Prepare RNase-free DNase I reaction buffer (1 mM NaCl, 10 mM MgCl₂, 20 mM Tris-HCl, pH 7.0 at 25°C) and make the final concentration of DNase I to 0.1 U/µl

• **General Protocol for culture cells (Trizol treated) :**

1. Lyse cells in Trizol® reagent or equivalent:

• **Cell grown in monolayer:**

- a. Remove growth media.
- b. Add 0.3 – 0.5 mL of Trizol® reagent per $0.5 - 5 \times 10^6$ cells directly to the culture dish to lyse the cells.
- c. Pipet the lysate up and down several times to homogenize.

• **Cells grown in suspension:**

- a. Collect approximately $0.5 - 5 \times 10^6$ cells and pellet cells by centrifugation at 300g for 5 minutes. Discard the supernatant.
Note: Do not wash cells before addition of Trizol Reagent to avoid mRNA degradation.
- b. Add 0.3 – 0.5 mL of Trizol® reagent to the cell pellet.
- c. Pipet the lysate up and down several times to homogenize.

IMPORTANT! Do not lyse too many cells in reduced Trizol® reagent volumes. This will make cell lysis incomplete and lead to low RNA yield and purity.

2. Add a volume of 70 - 100% RNase-free ethanol equal to the Trizol® reagent volume used in step 1 and mix thoroughly.

3. Place a FSRB Mini Column to a Collection Tube and transfer the ethanol added sample mixture (including any precipitate) to the FSRB Mini Column. Centrifuge at full speed for 1 min, discard the flow-through and return the FSRB Mini Column back to the Collection Tube.

Note: If the volume of the ethanol added sample mixture exceeds the capacity of the FSRB Mini Column, repeat step 3 with the remaining volume.

4. **Optional step:** DNase I digestion to eliminate genomic DNA contamination, follow the steps 4 a-d. Otherwise, proceed to step 5 directly.

- 4a. Add 250 µl of Wash Buffer 1 to the FSRB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.
- 4b. Add 60 µl of RNase-free DNase 1 solution (0.1U/ul) to the membrane center of the FSRB Mini Column. Place the column on the benchtop for 15-20 min.
- 4c. Add 250 µl of Wash Buffer 1 to the FSRB Mini Column, centrifuge at full speed for 1min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.
- 4d. After DNase 1 treatment, proceed to step 6 (do not perform step 5).

5. Add 500 µl of Wash Buffer 1 to the FSRB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.

6. Add 750 µl of Wash Buffer 2 to the FSRB Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the FSRB Mini Column back to the Collection Tube.

Note: Make sure that ethanol has been added into Wash Buffer 2 when first use.

7. Repeat step 6 for one more washing.

8. Centrifuge the FSRB Mini Column at full speed for an additional 3 min to dry the FSRB Mini Column.

IMPORTANT! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

9. Place the FSRB Mini Column to an Elution Tube (provided, 1.5 ml microcentrifuge tube).

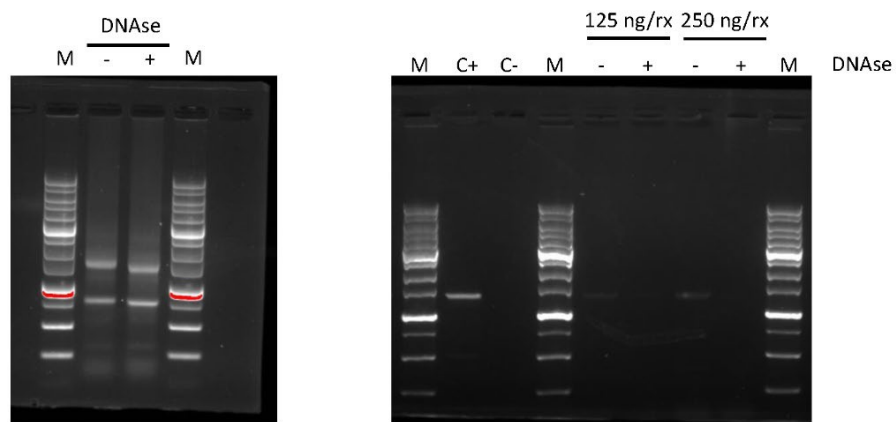
10. Add 40 - 100 µl of RNase-free ddH₂O to the centre of the FSRB Mini Column membrane.

11. Stand the FSRB Mini Column for 1 min.

IMPORTANT! Do not elute the RNA using less than suggested volume (< 40 µl). It will lower the RNA yield.

12. Centrifuge the FSRB Mini Column at full speed for 1 min to elute RNA.

13. Total RNA is ready for downstream application or to be stored at -80°C.



Troubleshooting:

Problem	Possible reasons	Solutions
Little or no RNA eluted	RNA remains on the column	• Repeat elution. • Pre-heat DEPC-water to 70° C prior to elution. • Incubate for 5 min with water prior to elution.
Degraded RNA	Source	• Follow protocol closely, and work quickly.
	RNase contamination	• Ensure not to introduce RNase during the procedure. • Check buffers for RNase contamination.
Problem in downstream applications	Salt carry-over during elution	• 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	• Use provided RNase-free water. • Use 10 mM Tris-HCl, not the DEPC water to dilute the sample before measuring purity.

• **General Protocol for Tissues total RNA and miRNA(Trizol treated):**

1. **Tissues:** Weight from 10 mg up to 30 mg of tissue sample to a microcentrifuge tube (not provided)
2. Add **1 ml** Trizol into the 1,5 ml tube to lyse sample.

Tissues:** Homogenize the tissue by using a rotor-stator homogenizer or we recommend first grinding the tissue using a mortar and pestle or micropestle, or a potter, you can also use a handhend homogenizer (cat.#FSHZ-02) with a stainless still blade of 6mm

3. Stand the homogenates for 5 mins at room temperature.
4. Add 200 µl chloroform into the tube, vortex vigorously for 2 mins.
5. Centrifuge for 3 mins. Transfer the upper phase carefully into a new 1,5 ml centrifuge tube.

• **IMPORTANT!** Avoid picking up any of the interphase or organic layer when transferring the aqueous phase (upper phase).

6. Add RNase-free 96~100% **ethanol** (1.5× volume of upper phase) to the upper phase and mix thoroughly.
 - If the upper phase volume is 500 µl, add 750 µl of RNase-free 96~100% ethanol to upper phase
7. Place a Column to a Collection Tube and transfer 900 µl of the sample mixture to the Column. Centrifuge for 1 min, discard the flow-through and return the Column back to the Collection Tube.
8. Repeat **step 7** for remaining sample mixture.
9. **(Optional step)** On-column DNase I digestion, if eliminating gDNA contamination in RNA sample is required, proceed the "On-column DNase I digestion".

10. Add 700 µl of Wash Buffer 1 to the Column, centrifuge for 1 min. Discard the flow-through and place Column back to the Collection Tube.

Note: This wash step (10.) is for the column containing large RNA only. For the column containing small RNA, skip this step and proceed directly to Step 11

11. Add 750 µl of Wash Buffer 2 (ethanol contained) to the Column, centrifuge for 1 min. Discard the flow-through and place the Column back to the Collection Tube.
12. Add 750 µl Wash Buffer 2 (ethanol contained) to the Column. Centrifuge for 2 mins to dry the membrane directly. Discard flow-through and Collection Tube.

13. Place the Column to an Elution Tube, then add 30 µl of RNase-Free Water to the membrane center of the Column. Stand the Column for 5 mins.

IMPORTANT! For effective elution, make sure that RNase-Free Water is dispensed onto the membrane center and absorbed completely.

14. Centrifuge the Column for 3 mins to elute RNA.

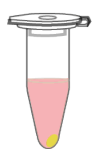
15. Store RNA at -70°C.

Troubleshooting:

Suggestions		Solutions
We suggest to use a rotor-stator homogenizer	If a homogenizer is not available	we recommend first grinding the tissue using a mortar and pestle or micropestle
use a handhend homogenizer	(cat.#FSHZ-02) with a stainless still blade of 6mm	no large visible tissue fragments must remain
we recommend reducing the tissue input amount to ensure sufficient lysis		The goal is to ensure that the tissue is thoroughly disrupted,

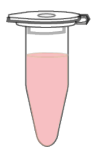
Tissue Samples

Tissue Sample

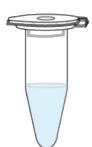


- Lysis the sample in 1 ml **Trizol**.
- Stand the sample for 5 mins at room temperature.

Centrifuge
18,000 ×g, 3 mins



- Add 200 µl chloroform.
- Vigorous vortexing for 2 mins.



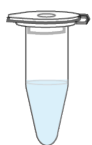
- Transfer upper phase.
- Add 0.54× ethanol (96~100%).

HE Column

Centrifuge
18,000 ×g, 1 min



- Transfer mixture to **Column**.
- Binding large RNA.
- Save the filtrate.



- Transfer the filtrate to new tube.
- Add 1.1× ethanol (96~100%).

Centrifuge
18,000 ×g, 1 min



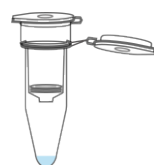
- Transfer mixture to **Column**.
- Binding small RNA.

Centrifuge
18,000 ×g, 1 min



- Add 700 µl **Wash Buffer 1** (ethanol contained) to **large RNA Column** and centrifuge
- Add 750 µl **Wash Buffer 2** (ethanol contained) and dry the column membrane. For **miRNA**

Centrifuge
18,000 ×g, 3 mins



- Elution (add 30 µl **RNase-Free Water**).
- Stand the column for 5 mins at room temperature.
- Obtain purified large RNA and small RNA.

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