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

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), 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.
- No need for chloroform, phase-separation or precipitation steps.
- 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 ug of Total RNA/column
- **Handling Time:** Within 30 minutes (20 min if DNAse treatment is not performed)
- **Expected yield:** 10 µg of total RNA per column
- **Format:** Spin Column
- **Minimum elution volume:** 40 ul

Storage Conditions

Stable for 1 year at room temperature.

Kit components	RE-040 100 preps	RE-041 200 preps	Store at
Wash 1 Buffer	60ml	2X 60ml	Store at room temperature (15~ 25°C) for 1 year.
Wash 2 Buffer conc.*	35 ml *	2X 35 ml *	
RNase-free Water	6 ml	2X 6 ml	
FSRB Mini Column	100 pieces	2X 100 pieces	
2.0 ml Collection Tube	100 pieces	2X 100 pieces	
1.5ml Elution Tube	100 pieces	2X 100 pieces	
DNAse I **	1 vial	1X 1 vial	
TriZol reagent	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/ul *

Required Material – not provided: DNAse I reaction buffer (1 mM NaCl, 10 mM MgCl2, 20 mM Tris-HCl, pH 7.0 at 25ºC)
**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 25ºC) and make the final concentration of DNase I to 0.1 U/µl

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• General Protocol:

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.

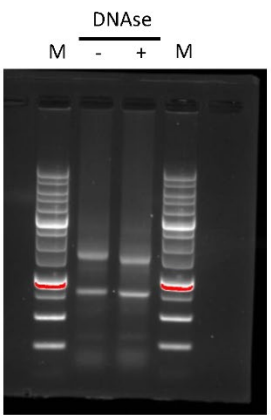
7.Repeat step 6 for one more washing.
8.Centrifuge the FSRB Mini Column at full speed for an additional 3 min to dry the FSRB Mini Column.

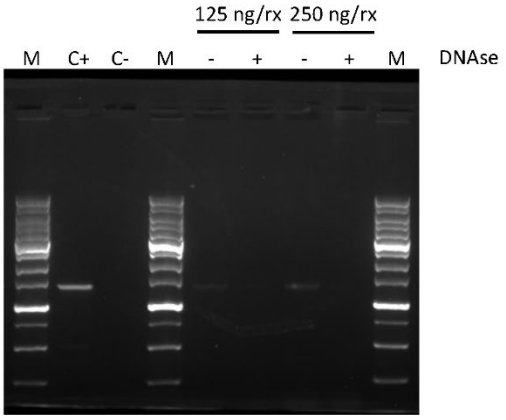
IMPORTANT! This step will avoid the residual liquid to inhibit subsequent enzymatic reaction.

9.Place the FSRB Mini Column to an Elution Tube (provided, 1.5 ml microcentrifuge tube).
10.Add 40 - 100 µl of RNase-free ddH_2O 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.
Abnomal 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.

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36 Terry Drive
Trevose, PA 19048 - USA