

High Efficiency (HE) RNA Extraction Cells & Tissues

RE-001-HE

The High Efficiency **RNA Extraction Cells & Tissues Mini Kit** is one of the most innovative RNA isolation kit available, designed for the easy, reliable, and rapid isolation of DNA-free RNA from a very small volume of cells (from 10^1 up to 10^7) and various tissue samples (up to 30 mg).

Format/Principle	Spin column (Silica matrix)
Binding Capacity	$\leq 150 \mu\text{g}$ NA/Column
Operation Time	<45 mins
Sample Size	≤ 30 mg Tissue $1 \times 10^1 - 1 \times 10^7$ Cultured Cell
RNA yield	$\leq 75 \mu\text{g}$
Elution Volume	30 μl

Highlights of HE Columns:

- Fast and streamlined extraction process...
- Delivering high-yield nucleic acid even from cells & tissues.
- The **HE Column technology** allow high yield total RNA from any sample

Kit components	RE-HE-001 (50 preps)
FSRB Buffer	30 ml
Wash Buffer 1	30 ml
Wash Buffer 2 ^a (concentrate)	20 ml
RNase-free Water	6 ml
Filter Column	50 pcs
HE Mini Column	50 pcs
HE Collection Tube	3X50 pcs
Elution Tube	50 pcs
Volume of Ethanol to add to Wash buffer 2	80 ml

■ Procedure Overview

Animal tissues



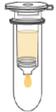
- Cut the tissue (up to 30 mg) into a 1.5 ml tube (Not provided).
- Add 550 μl β -ME-FSRB mixture.
- Homogenize using a homogenizer or Micropestle.
- Stand at room temperature for 5 mins.

Animal cells



Centrifuge
300 \times g, 5 mins

- Centrifuge to pellet the cells up to 1×10^7 .
- Discard the supernatant.
- Add 550 μl β -ME-FSRB mixture.
- Vortex for 1 min.



Centrifuge
18,000 \times g, 3 mins

- Transfer entire mixture into **Filter Column**.
- Transfer all the supernatant of the filtrate into a new 1.5 ml tube.

HE Column



Centrifuge
18,000 \times g, 3 mins

- Add 1 \times volume of RNase-Free 70% ethanol.
- Transfer up to 1000 μl of the sample mixture into the **HE Column** for RNA binding. (Repeat the step if the mixture volume exceeds 1000 μl .)



Centrifuge
18,000 \times g, 1 min

Centrifuge
18,000 \times g, 2 mins

- Add 500 μl **Wash Buffer 1** and centrifuge.
- Add 750 μl **Wash Buffer 2** (ethanol contained) and centrifuge
- Add 750 μl **Wash Buffer 2** (ethanol contained) and dry the column membrane.

Centrifuge

- Add 30 μl **RNase-Free Water**.
- Stand the column for 5 mins at room temperature.
- Obtain purified RNA.

Preparation Before Starting

1. Additional materials: β -mercaptoethanol (β -ME), RNase-Free 70% and 96~100% ethanol, 20-G needle with syringe (optional), and DNase I (optional).
2. (Optional) For long-term RNA storage, immerse the animal tissue in Reagent as instructed in the user manual.
3. (Optional) Prepare DNase I working solution following the user guide of DNase I Solution and make the final concentration of 0.25 U/ μl .
4. For a fresh preparation of β -ME-FSRB mixture, premix 5.5 μl of β -ME and 550 μl of FABR Buffer per sample before executing RNA extraction.
5. Caution: β -ME is hazardous to human health. Always perform procedures involving β -ME in a fume hood.
6. Add the indicated volume of ethanol (96~100%) into Wash Buffer 2, mix well, and store at room temperature.

FOR ANIMAL TISSUES

1. Add 550 μ l of β -ME-FSRB mixture to the tissue samples up to 30 mg.
 - Note: For RNA-rich or soft organ tissues (e.g., liver or kidney), limit the sample input to $\leq 10 \sim 15$ mg to avoid column overload.
2. Homogenize the sample using a homogenizer or Micropestle.
 - Note: If debris remains, pass the lysate through a 20-G needle syringe 10 times.
3. Incubate the sample at room temperature for 5 mins.
 - Note: If the lysate appears gel-like or stringy, reduce the input amount or split across multiple tubes.
4. Proceed to the General Protocol.

FOR CULTURE CELLS

1. Transfer up to 1×10^7 cultured cells to a 1.5 ml microcentrifuge tube (not provided).
2. Add 550 μ l of β -ME-FSRB mixture and lyse the cells by vortexing vigorously for 1 min.
3. Proceed to the General Protocol.

GENERAL PROTOCOL

- Note: All centrifugation steps should be performed at 18,000 \times g at room temperature.
1. Ensure the sample has been appropriately processed as instructed in the
 2. Place a Filter Column in an HE Collection Tube and transfer the entire mixture to the Filter Column.
 3. Centrifuge for 3 mins. Carefully transfer the supernatant of filtrate into a new a 1.5 ml microcentrifuge tube (not provided).
 - Note: Avoid pipetting any debris and pellets when transferring the supernatant.
 4. Measure the volume of the supernatant and add 1 \times volumes of RNase-Free 70% ethanol to the sample mixture. Mix thoroughly by vortexing.
 5. Place an HE Column in an HE Collection Tube. Transfer all the mixture (including any precipitate) into the HE Column. Centrifuge for 3 mins and discard the flow-through. Repeat this step until all mixture has been processed.
 6. (Optional) DNase I digestion. To eliminate genomic DNA contamination, follow the steps from a.
 - a. Add 250 μ l of Wash Buffer 1 to the HE Column, and centrifuge for 1 min. Discard the flow-through and place the HE Column in the HE Collection Tube.
 - b. Add 750 μ l of RNase-Free 70% ethanol to the HE Column, and centrifuge at 18,000 \times g for 1 min. Discard the flow-through and place the HE Column in the HE Collection Tube.
 - c. Add 60 μ l of RNase-Free DNase I solution (0.25 U/ μ l, not provided) to the membrane center of the HE Column. Place the column on the benchtop for 15 mins.
 - d. Add 250 μ l of Wash Buffer 1 to the HE Column, and centrifuge for 1 min. Discard the flow-through and place the HE Column in the HE Collection Tube.
 - e. Proceed to step 8.
 7. Add 500 μ l Wash Buffer 1 to the HE Column. Centrifuge for 1 min then discard the flow-through.
 8. Add 750 μ l Wash Buffer 2 (ethanol contained) to the HE Column. Centrifuge for 1 min then discard flow-through.
 9. Add 750 μ l Wash Buffer 2 (ethanol contained) to the HE Column. Centrifuge for 2 mins to dry the membrane directly. Discard flow-through and HE Collection Tube.
 - Important step! Don't let the tip of the column touch the flow-through. If in doubt, spin again to remove all residual ethanol.
 10. Place the HE Column in an Elution Tube, then add 30 μ l RNase-Free Water directly onto the membrane. Stand the HE Column for 5 mins.
 - Important step! For effective elution, ensure that the elution solution is dispensed onto the membrane center and absorbed completely.
 11. Centrifuge for 2 mins to elute the RNA.