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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).

Highlights of HE Columns:

- Fast and streamlined extraction process…
- Delivering high-yield nucleic acid even from

The **HE Column technology** allow high yield total RNA from any sample

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

Format/Principle	Spin column (Silica matrix)
Binding Capacity	≤150 µg RNA/Column
Operation Time	<45 mins
Sample Size	≤30 mg Tissue 1x 10^1 - 1 × 10^7 Cultured Cell
RNA yield	≤75 µg
Elution Volume	30 µl

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.

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FOR ANIMAL TISSUES

1. Add 550 µl 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 ≤10~15 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 µl 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 ×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× 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 µl 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 µl of RNase-Free 70% ethanol to the HE Column, and centrifuge at 18,000 ×g for 1 min. Discard the flow-through and place the HE Column in the HE Collection Tube.
   c. Add 60 µl of RNase-Free DNase I solution (0.25 U/µl, not provided) to the membrane center of the HE Column. Place the column on the benchtop for 15 mins.
   d. Add 250 µl 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 µl Wash Buffer 1 to the HE Column. Centrifuge for 1 min then discard the flow-through.
8. Add 750 µl Wash Buffer 2 (ethanol contained) to the HE Column. Centrifuge for 1 min then discard flow-through.
9. Add 750 µl 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 µl 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.