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Product information

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EZ-10 Spin Column Total RNA Mini-Preps Kit

Catalog #: BS1361
Size: 50 preps
Storage: 4°C*

Product Description:

The EZ-10 Column Total RNA Mini-Prep Kit allows efficient purification of total RNA from various samples. Total RNA is easily purified from animal or human cells and tissues using a simple spin format. This kit simplifies total RNA isolatoon by combining the stringency of guanidine-isothiocyanate lysis with the speed and purity of silica-based purification. Samples are first lysed and then homogenized. Ethanol is added to the lysate to provide optimal binding conditions. The lysate is then loaded onto the EZ-10 column with a silica membrane. While the RNA binds to the silica membrane, all proteins and other components are removed in the flow-through. Remaining contaminants and salts are efficiently washed away. The purified RNA eluted in RNase-free water has OD260/OD280 ratios of 1.9-2.1 (measured in 10 mM TrisHCl, pH 7.5) and is ideal for use in most downstream applications including Northern blotting, RT-PCR, Quantitative PCR, Poly (A) RNA selection and Array analysis.

Contents:

Description	Size
Buffer RLT	25ml
Buffer RW	30ml
Universal RPE Solution	12ml
Rnase-free Water	5ml
Ez-10 Spin Column	50 pc
2 ml Collection Tube	50 pc
Protocol	1 pc

Procedure:

1. Sample preparation:

A. Adherent cells: Do not use more than $1X10^7$ cells. Cells can be either lysed directly in the cell culture petri-dish or trypsinized and collected as a cell pellet prior to lysis. For direct lysis of cells grown in a monolayer, add 0.45 ml Buffer RLT to the cell-culture dish. Collect the lysate with a rubber policeman. Pipet the lysate into a microcentrifuge tube (not supplied). Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 2.

To trypsinize and collect cells: Determine the number of cells. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, and add 0.1–0.25% trypsin in PBS. After the cells detach from the dish or flask, add medium (containing serum to inaactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 300 x g for 5 min. Completely aspirate the supernatant. Add 0.45 ml Buffer RLT per 5 cm^2 cultured cell. Mix gently by pipetting up and down several times.

Procedure Continued:

B. Suspension cells: Collect cells by centrifuge, discard the supernatant. Loosen the cell pellet thoroughly by flicking the tube. Add 0.45 ml Buffer RLT for 1-5 × 10^6 cells, mix gently by pipetting. The amount of cells should not exceed 1 × 10^6 for fibroblasts or carcinoma cells.
C. Fresh Tissue: Cut the tissue into pieces and grind to fine powder in liquid nitrogen. Add 0.45 ml Buffer RLT for 25-50 mg tissue, homogenate for 30 sec using a rotor-stator homogenizer. Alternavely, one can pass the lysate at least 5 times through a blunt 20-Gauge needle (0.9mm diameter) fitted to an RNase-free syringe. Proceed to step 2.

2. Add 1/2 volume of ethanol, mix by inverting the tube. Do not centrifuge.

3. Transfer the solution including any precipitate that may have formed, to the spin column placed in a 2ml collection tube. Centrifuge at 12,000 × g for 30 sec at room temperature, discard the flow-through.

4. Add 0.5 ml of RW Soluon to the column, centrifuge at 12,000 × g for 30 sec at room temperature, discard the flow-through.
Note: If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same EZ-10 spin column. Discard the flow-through after each centrifugation.

5. Add 0.5 ml of Universal RPE Solution to the column, centrifuge at 12,000 × g for 30 sec at room temperature, discard the flow-through.
Note: Universal RPE Solution is supplied as a concentrate. Before use, add 48ml of 96-100% ethanol to 12 ml concentrated universal RPE solution and mix well.

6. Centrifuge the column at 12,000 × g for 30 sec at room temperature.
Note: This step is very important to remove the residual ethanol thoroughly.

7. Place the column to a new 1.5 ml centrifuge tube; add 50 µl RNase-free Water. Keep at room temperature for 2 minutes. Centrifuge at 12,000 × g for 30 sec at room temperature, save the eluted RNA soluon at -80°C.

Note:

Care must be taken when working with RNA. It is important to maintain an RNase-free environment, starting with RNA sample preparation and continue through.