PRODUCT INFORMATION

EZ-10 Spin Column Total RNA Mini-Preps Kit

Product information for BS1361:

Kit Components:

Components	BS1361, 50 Preps
Buffer RLT	25 ml
Buffer RW	30 ml
Universal RPE Solution	12 ml
RNase-free Water	5 ml
EZ-10 Spin Column	50
2 ml Collection Tube	50
Protocol	1

Storage

The kit is valid for 2 year at 4°C.

Note:

- Universal RPE Solution is supplied in a concentrated form, before use, add 48 ml 96-100% ethanol to 12 ml concentrated universal RPE solution and mix well.
- 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

purification and analysis. Use RNase free tubes, tips, gels. Wear gloves at all times.

Introduction

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 isolation 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. RNA binds to the silica membrane, all protein and other components are removed in the flow-through. Remaining contaminants and salts are efficiently washed away. Purified RNA is eluted in RNase-free water has OD_{260}/OD_{280} ratios of 1.9-2.1 (measured in 10 mM Tris-HCl, 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.

Procedures

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 inactivate 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.

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 cell.

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. Alternatively, 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 Solution 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. Ensure that ethanol is added to Buffer RPE before use.

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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 solution at -80 °C

PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY!
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