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Total RNA Purification using illustra™ RNAspin Mini and Midi RNA Isolation Kits

Extracted from **Nucleic Acid Sample Preparation for Downstream Analyses**, GE Healthcare, 2007

The following protocol summarizes use of the illustra™ RNAspin Mini and Midi RNA Isolation Kits. Most of the materials and preparations are the same for the RNAspin 96 Kit, but the RNAspin 96 Kit includes one additional buffer for dilution of lysate and for washing. In addition, collection trays are provided with this latter kit. See **Chapter 9** for the protocol for RNAspin 96.

Materials

Kits provide illustra™ RNAspin columns, filter units, buffers, lyophilized DNase I, DNase reaction buffer, RNase-free water, and collection tubes

Gloves

RNase-free tubes and other plasticware

β-mercaptoethanol

For bacteria: lysozyme (0.2 mg/ml final concentration for Gram-negative cells; 2 mg/ml final for Gram-positive cells) in TE buffer, pH 8

For yeast: zymolase/lyticase in sorbitol/EDTA buffer (1 M sorbitol, 100 mM EDTA)

Optional for all samples: 0.9 mm needle (20-gauge) and syringe

Advance preparation

Dissolve DNase I in RNase-free water.

Avoid vigorous mixing of the DNase I enzyme because it is sensitive to mechanical agitation and will be inactivated.

Add ethanol to buffer RA3 concentrate.

Protocol

1. Disrupt sample and lyse cells

a. Disrupt tissue or do other sample pretreatment if needed. Disrupt animal tissue using liquid nitrogen and a mortar and pestle or other suitable method. Immediately proceed to Step 2 to lyse cells. Harvest cultured cells by centrifugation. Immediately proceed to Step 2 to lyse cells. Pretreat harvested bacterial cells with lysozyme. Pretreat harvested, cultured yeast cells with lyticase or zymolase. Centrifuge and isolate spheroplasts.

b. Lyse cells. Add lysis buffer RA1 and β-mercaptoethanol. Vortex vigorously. The chaotropic salts in the lysis buffer break open the cells and inhibit RNases. β-mercaptoethanol helps to inhibit RNases and break up RNA-protein complexes.

2. Filter lysate

Reduce viscosity and clear the lysate by centrifugation through an RNAspin filter unit.

3. Adjust RNA binding conditions

Add 70% ethanol to the cleared lysate and apply to silica column.

4. Bind RNA

The chaotropic salt in the lysis buffer plus the ethanol promote binding of RNA > 200 nucleotides long to the silica membrane. Denatured proteins are collected in the flowthrough following centrifugation. After the addition of ethanol, a stringy precipitate may become visible. This will not affect RNA isolation. Be sure to load all the precipitate onto the column as described in step 5.

5. Desalt the membrane

Add desalting buffer to wash away salts.

Maximal loading capacity of RNAspin Mini column is 750 µl. Repeat the procedure if larger volumes are to be processed.

6. Add DNase I

Add RNase-free DNase to digest membrane-bound DNA. Incubate briefly at room temperature.

7. Wash and dry

Wash the column to inactivate DNase, then wash with a low-salt buffer containing ethanol to remove residual salts and other contaminants. Dry the membrane.

8. Elute purified total RNA

Elute purified total RNA using RNase-free water. Immediately place on ice or freeze.

Materials

Product #	Description	Add to Cart
GE25-0500-70	RNAspin Mini (20) Cytiva, 25-0500-70, sufficient for 20 preparations	pricing
GE25-0500-71	RNAspin Mini 50 Cytiva, 25-0500-71	pricing
GE25-0500-72	RNAspin Mini 250 Cytiva, 25-0500-72	pricing