Fast Tissue RNA Purification Kit

Catalog No.: EZB-RN5

Description

The *EZ*Bioscience® Fast Tissue RNA Purification Kit provides a simple, reliable, and rapid method for isolating high-quality total RNA from animal tissues and cells, providing high sample processing capability to deal with difficult specimens. Genomic DNA contamination is effectively removed using DNase treatment. The purified total RNA is ready to use and is ideally suitable for downstream applications that are sensitive to low amounts of DNA contamination, such as quantitative, real-time RT-PCR. The purification RNA can also be used in other applications including: RT-PCR, Northern blotting, nuclease protection assays, cDNA library preparation after poly (A)+ selection, and so on. Samples are first lysed and homogenized in a highly denaturing guanidine-isothiocyanate-containing buffer, which immediately inactivates RNases to ensure isolation of intact RNA. After homogenization, ethanol is added to the lysate to provide appropriate binding conditions for RNA, and the sample is then applied to an RNA spin column, where total RNA binds to the membrane. Genomic DNA is removed by DNase treatment and Contaminants are effectively removed by subsequent washing. The purified total RNA is then eluted with Elution Buffer.

Component

Component	EZB-RN5 (100 Preps)
Lysis Buffer	65 ml
Wash Buffer 1*	13 ml
Wash Buffer 2*	13 ml
Elution Buffer	25 ml
gDNA Remover	220 µl
Spin Columns (with Collection Tubes)	100 Preps

*: Before using for the first time, add 52 ml 100% ethanol to the Wash Buffer and mix thoroughly.

Storage

Store the gDNA Remover at -20° C. Store other components at room temperature (When using these buffers, be careful to avoid contamination). The Lysis Buffer is suggested to be stored at 4°C when the room temperature is >25°C, or for long-term storage.

Protocol

The Lysis Buffer should be restored to room temperature before using.

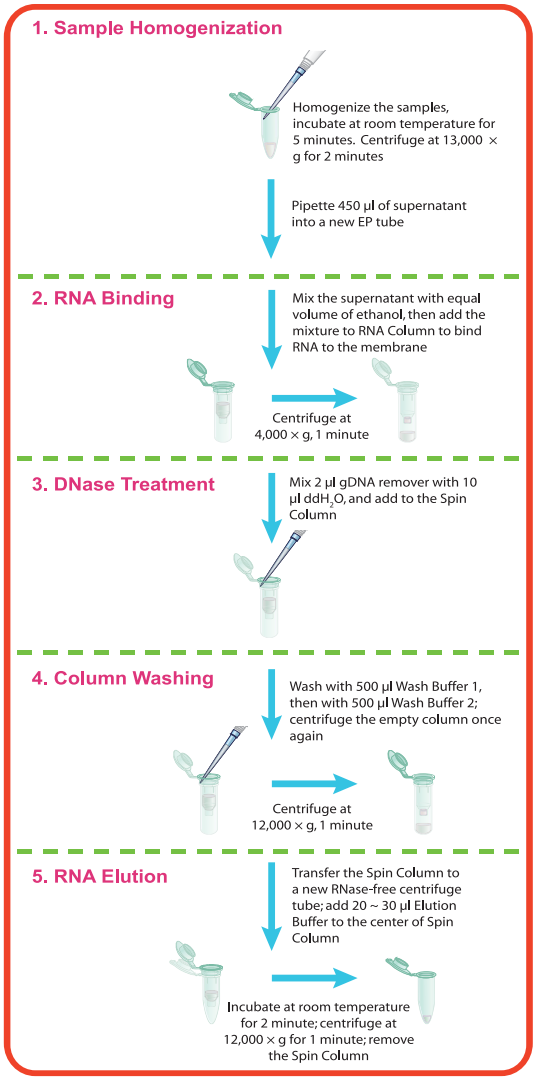
Sample Homogenization

The suggested tissue weight according to tissue types:

Tissue types	Adipose tissue	Liver	Tumor, embryos, heart, kidney, spleen, pancreas, lung, brain, eye
Weight (mg)	30~50	5~ 10	5~ 15

1. Add 600 µl of Lysis Buffer to a 1.5 ml centrifuge tube, then place 5 ~ 50 mg tissue (cell samples can be lysed in culture dishes directly).
2. Homogenize the tissue with a pestle or rotor-stator homogenizer. Incubate at room temperature for 5 minutes.
3. Centrifuge the lysate at 13,000 × g for 2 minutes, then carefully transfer 450 µl of supernatant to a new RNase-free 1.5 ml tube (Don't aspirate more, and avoid aspirating the residue at the bottom of the centrifuge tube). For tissues with high fat content, after centrifugation, the upper layer will be seen as an oil layer. Insert the pipette tip directly into the middle layer and aspirate the supernatant (about 400 µl) of the middle layer into a new 1.5 ml EP tube.

Experimental Procedure Overview:



RNA Binding

4. Add equal volume of 100% ethanol to the supernatant, then invert the centrifuge tube for several times or pipette up and down for 10 times to mix thoroughly (if there may be precipitates at this step, it is necessary to disperse the precipitates), and transfer the mixture to the Spin Column. Centrifuge at 4,000 × g for 1 minute. If there is liquid remained in the column, centrifuge at 12,000 × g for 1 minute to eliminate the liquid.

DNase Treatment

5. Mix 2 μl gDNA Remover with 10 μl ddH_2O, and add to the membrane in the center of t he Spin Column. Incubate at room temperature for 5 minutes to degrade the residual genomic DNA.

Column Washing

6. Add 500 μl of Wash Buffer 1 to the Spin Column. Centrifuge at 12,000 × g for 1 minute to wash the spin column membrane. Discard the flow-through.
7. Add 500 μl of Wash Buffer 2 to the Spin Column, Centrifuge at 12,000 × g for 1 minute to wash the spin column membrane. Discard the flow-through.
8. Pour off the flow-through of the collection tube, then place the Spin Column back into the collection tube, centrifuge at 12,000 × g for 1 minute to eliminate any possible carryover of Wash Buffer.
9. Don't need to pour off the li quid of the collection tube, directly transfer the Spin Column to a new RNase-free 1.5 ml centrifuge tube, open the lid and incubate at room temperature for 2 minutes.

RNA Elution

10. Add 20 ~ 30 μl of Elution Buffer to the membrane in the center of the Spin Column and incubate at room temperature for 2 minutes.
11. Centrifuge at 12,000 × g for 1 minute to elute RNA (transfer the eluate back to the column, incubate for 3 minutes and centrifuge once more will get more RNA).
12. Discard the column , put RNA on ice immediately and determine the RNA concentration (**Note:** be sure to mix RNA thoroughly before determi ning the concentration of RNA), then do the following ex periment directly, or store the RNA at -80°C until needed.

Representative Results

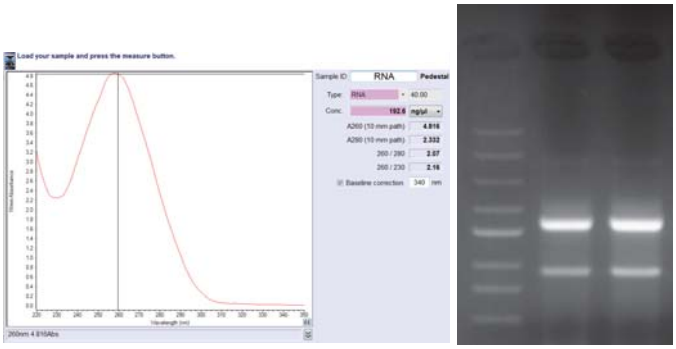


Figure 1. RNA isolated from Adipose Tissues using ***EZ*Bioscience**® Fast Tissue Purification Kit M: 250bp DNA Ladder(left); The RNA purified using the kit, detected by Nanodrop.

The data from Figure 1. indicate that, Fast Tissue RNA Purification Kit can completely replace the TRIzol method in RNA purification.

Trouble shooting

The quantity of RNA purified by th is Kit is too small, or the qPCR results is not so good, the Ct value is too large, or some specific genes can not be amplified normally.

Suggestions:

a. Examine whether any of the reagents in the Kit is contaminated: use a new Kit as positive control to confirm whether the reagents are contam inated (If the reagents are contaminated, the results between these two will be different, obviously). Therefore, **it is recommended to divide the reagents into 3 ~ 4 parts, each part in a 15 ml/50 ml centrifuge tube.** Be careful when taking the reagents out of the bottles to avoid of contamination.

b. The gene specific primers for qPCR should be divided to aliquots and stored at -20°C, to decrease the possibility of degradation or contamination.

C. Examine whether the experiment is carried out correctly.

e.g.:

1. During the whole process of RNA purification by this Kit, **it must be manipulated at room temperature but not on ice** (until the RNA is eluted after the centrifuge), to avoid of blocking the membrane in the Spin Column by the water-insoluble substances formed during ice cooling.

2. Before using this Ki t for the first time, **add 52 ml 100% ethanol to the Wash Buffer 1 and Wash Buffer 2 respectively,** and mix thoroughly.

3. Before loading the column, the tissue lysate must be centrifuged at 13000 g for 2 minutes, and the supernatant should be aspirated into a new EP tube and mixed with 100% ethanol, otherwise the column will be clogged.

4. After washing the sample, the column should be taken out carefully to avoid contacting the bottom of column with liquid.

5. When doing the step of RNA bind ing and RNA elution, after centrifuge, **transfer the eluate back to the column and repeat the centrifuge once again will get more RNA.**