Tissue RNA Purification Kit (for Adipose Tissue)

Catalog No.: EZB-RN001A

Description

The *EZ*Bioscience® Tissue RNA Purification Kit (for Adipose Tissue) integrates phenol/guanidine-based sample lysis and silica-membrane purification of high-quality total RNA from animal tissues and cells with high fat content. Samples are first lysed and homogenized in Lysis Buffer, which immediately inactivates RNases to ensure isolation of intact RNA. After addition of chloroform, the homogenate is separated into aqueous and organic phases by centrifugation. RNA partitions to the upper, aqueous phase while DNA partitions to the interphase and proteins to the lower, organic phase or the interphase. The upper, aqueous phase is collected, and ethanol is added to provide appropriate binding conditions. The sample is then applied to the RNA Spin Column, where the total RNA binds to the membrane, and contaminants are effectively washed away. Finally, high-quality RNA is eluted in Elution Buffer. The purified total RNA is suitable for use in a variety of downstream applications, including: RT-PCR, RT-qPCR, RNA-seq, Northern blotting, Poly A+ RNA selection, Microarray analysis, and so on.

Components

Components	EZB-RN001A (100 Preps)
Lysis Buffer	60 ml
Wash Buffer 1*	13 ml
Wash Buffer 2*	13 ml
Elution Buffer	25 ml
Spin Columns (with Collection Tubes)	100 Preps

*Before using for the first time, add 52 ml of 100% ethanol to the Wash Buffer 1 and Wash Buffer 2 separately.

Storage

Store the Lysis Buffer at 2 ~ 8°C, protected from light. Store other components at room temperature (when using these buffers, be careful to avoid contamination). Dividing the Elution Buffer into small aliquots upon reception is suggested.

Protocol

Sample Homogenization

The suggested tissue weight according to tissue types:

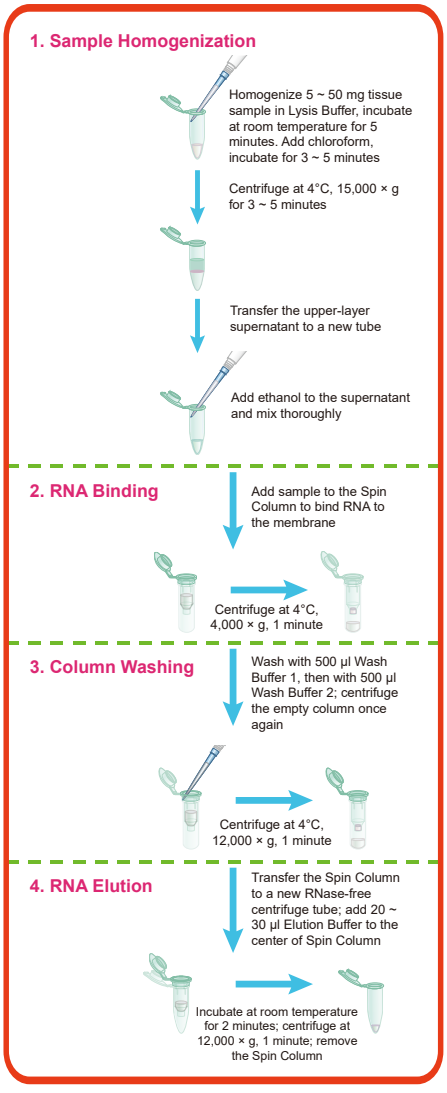
Tissue types	Adipose tissue	Liver	Tissues with high fat content
Weight (mg)	30~ 50	5~ 10	30~ 50

1. Place 5 ~ 50 mg tissue in a 1.5 ml centrifuge tube. Add 500 µl of Lysis Buffer (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. Add 150 µl chloroform to the tissue lysate, mix by pipette and hand-shaking. Incubate at room temperature for 3 ~ 5 minutes.
4. Centrifuge at 15,000 × g, 4°C for 3 ~ 5 minutes. Then the mixture separates into three phases. Transfer the upper-layer supernatant (about 200 µl) to a new RNase-free 1.5 ml centrifuge tube (be careful to avoid disturbing the middle or bottom layer).

RNA Binding

5. Add equal volume of 100% ethanol to each volume of supernatant for mRNA and lncRNA purification. Add 1.6 volume of 100% ethanol to each volume of supernatant for microRNA and circRNA purification.
6. Invert the centrifuge tube for several times or pipette up and down for 10 times to mix thoroughly, and transfer the sample to the Spin Column. Centrifuge at 4,000 × g, 4°C for 1 minute. Pour off the liquid.

Experimental Procedure Overview



Column Washing

7. Add 500 µl of Wash Buffer 1 to the column. Centrifuge at

12,000 × g, 4°C for 1 minute (be careful to avoid contacting the bottom of the column with the liquid when taking out of the column). Pour off the liquid.

8. Add 500 µl of Wash Buffer 2 to the column. Centrifuge at 12,000 × g, 4°C for 1 minute.
9. Pour off the liquid and eliminate the residual liquid using towel paper. Place the empty column back on the collection tube and centrifuge at 12,000 × g, 4°C for 1 minute.
10. Leave the liquid in the collection tube, directly transfer the column to a RNase-free 1.5 ml centrifuge tube, open the lid and keep in the air for 2 minutes.

RNA Elution

11. Add 20 ~ 30 µl of Elution Buffer to the center of the column and incubate at room temperature for 2 minutes.
12. Centrifuge at 12,000 × g, 4°C for 1 minute (transfer the eluate back to the column, incubate for 3 minutes and centrifuge once more will get more RNA).
13. Discard the column，determine the RNA concentration, do the following experiment with the purified RNA, or store the RNA at -80°C until needed.

Representative Results

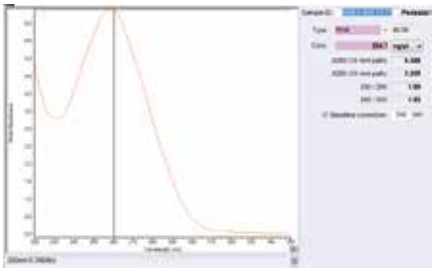


Figure 1. The RNA purified by this kit from adipose tissue was examined by Nanodrop, the concentration and purity(assessed by OD 260/280 and OD 260/230) was shown on the left.

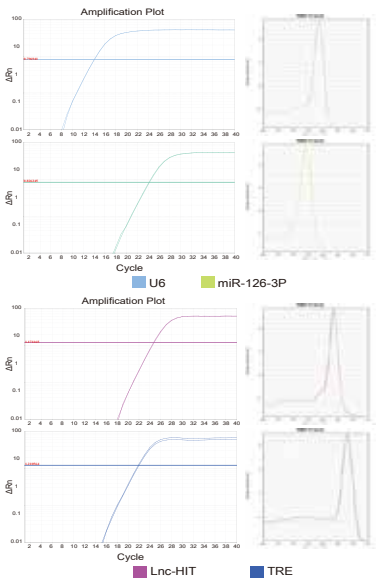


Figure 2. microRNA and lncRNA isolated from 293T cells are detected. These results showed that, this *EZ*Bioscience® Tissue RNA Purification Kit (for Adipose Tissue) can be used for microRNA and lncRNA purification and achieved good performance.

Trouble shooting

The quantity of RNA purified by this Kit is too small, or the qPCR result 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 contaminated (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 contamination.

b. The gene specific primers for qPCR should be aliquoted 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 (unless specially required) 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 Kit for the first time, **add 52 ml 100% ethanol to each bottle of the Wash Buffer** and mix thoroughly.
3. The tissue pieces must be weighed before RNA extraction. The weight of tissue should be less than 50 mg/sample. For tissues rich in RNA (such as liver), the amount of tissue should be reduced to no more than 10 mg/sample.
4. Transfer the upper-layer supernatant to a new tube after phase separation. **Avoid transferring any of the middle or bottom layer into the pipette when removing the upper-layer**.
5. **Add equal volume of ethanol to the supernatant** and mix thoroughly, then load onto the column.
6. After each column washing, the column should be taken out carefully to avoid of contacting the bottom of column with liquid.
7. The column must be dried in air for 2 minutes after the centrifugation of empty column.
8. When doing the steps of RNA binding and RNA elution, **load the flow-through of centrifugation to the column and repeat the centrifugation once again will get more RNA.**