EZ-press DNA & RNA Purification Kit

Catalog No.: B0009

Description

The *EZ*Bioscience® EZ-press DNA & RNA Purification Kit provides a simple, reliable, and rapid method for simultaneously isolating high-quality genomic DNA and total RNA from a wide variety of sources, without the need for toxic substances such as phenol or chloroform. The EZ-press DNA & RNA Purification Kit can be used with cultured cells and animal tissues. The purified genomic DNA is suitable for genotyping and other DNA analyses using PCR or DNA sequencing. And the purified total RNA is suitable for use in a variety of downstream applications, including: RT-PCR, RT-qPCR, Northern blotting, nuclease protection assays, cDNA library preparation after poly (A)+ selection, RNA sequencing and so on. Biological samples are first lysed and homogenized in a strong denaturant containing buffer, which immediately inactivates RNases to ensure isolation of intact RNA. After homogenization, the lysate is passed through a DNA binding column which traps the DNA on the column, while RNA can pass through the column with the flow-through (FT1). The DNA binding column is washed and then the genomic DNA is eluted by the Elution Buffer. On the other hand, ethanol is added to the RNA containing flow-through (FT1) from the first column. The mixture is then passed through an RNA binding Spin Column to which the RNA binds. Impurities are effectively removed by subsequent washing. The purified total RNA is then eluted by Elution Buffer and may be used in a variety of downstream applications.

Components

Components	B0009 (50 Preps)
Lysis Buffer	30 ml
Wash Buffer 1*	13 ml
Wash Buffer 2*	13 ml
Elution Buffer	25 ml
Spin Columns for DNA (with Collection Tubes)	50 Preps
Spin Columns for RNA (with Collection Tubes)	50 Preps

*Before using for the first time, add 20 ml of 100% ethanol to the Wash Buffer 1 and add 52 ml of 100% ethanol to the Wash Buffer 2.

Storage

Divide the Elution Buffer into small aliquots upon reception. Store all components at room temperature (When using these buffers, be careful to avoid of contamination).

Protocol

Sample Homogenization

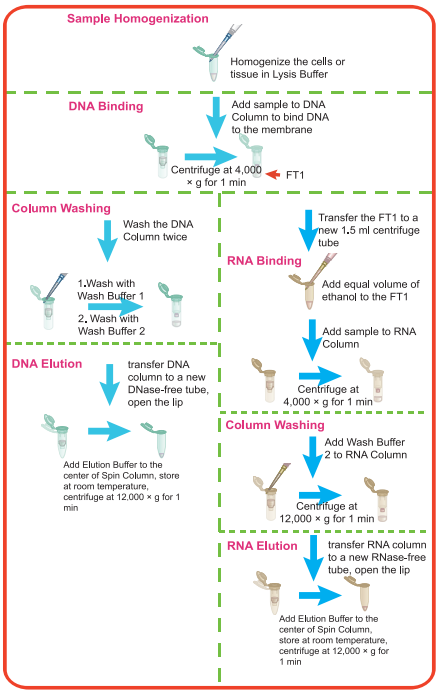
1A. For adherent cells ≤ 3 × 10^6/sample:

a) Remove the growth medium from the cells. Wash cells with appropriate volume of PBS.

b) Add 500 µl Lysis Buffer, pipette up and down for 30 times to lyse the cells or place on a shaker for 5 minutes, then pipette 10 times to fully lyse the cells

1B. For cultured suspension cells or adherent cells more than 3 × 10^6/sample:

Experimental Procedure Overview:



a) (For adherent cells only, for suspension cells, start at step b) Detach cells using the sub-culturing method routinely employed in your laboratory. Pellet 1 × 10^6 ~ 3 × 10^6 cells in a 1.5 ml centrifuge tube by centrifugation at 500 × g for 3 ~ 5 minutes.

b) Completely remove the supernatant by aspiration.

c) Add 500 µl Lysis Buffer. Vortex for 10 seconds at high speed to completely lyse the cells.

1C. For animal tissues:

a) Take no more than 5 mg tissue into a new RNase-free 1.5 ml centrifuge tube. Add 500 µl of Lysis Buffer.

b) Homogenize the tissue with a pestle or rotor-stator homogenizer.

c) After homogenization, incubate at room temperature for 5 minutes. Centrifuge at 12,000 × g for 1 minutes.

2. Transfer the cell lysate or the supernatant of the above-mentioned tissue lysate to the DNA Spin Column. Centrifuge at 4,000 × g for 1 minute. Transfer flow-through FT1 in the collection tube to a new RNase-free 1.5 ml centrifuge tube for RNA extraction. Then put the collection tube back into the DNA spin column.

DNA Column Washing

3. Add 500 µl Wash Buffer 1 to the DNA Spin Column, centrifuge at 12,000 × g for 1 minute. Discard the flow-though.
4. Add 500 µl Wash Buffer 2 to the DNA Spin Column, centrifuge at 12,000 × g for 1 minute. Discard the flow-though.
5. Put the collection tube back into the DNA Spin Column, centrifuge at 12,000 × g for 1 minute.
6. Don't need to pour off the liquid, directly transfer the column to a new 1.5 ml centrifuge tube. Open the lid and

keep in the air for 2 minutes.

DNA Elution

7. Add 30 ~ 50 µl Elution Buffer to the membrane in the center of the DNA Spin Column, and incubate at room temperature for 2 minutes.
8. Centrifuge at 12,000 × g for 1 minute. Discard the Spin Column, determine the DNA concentration, do the following experiment with the purified DNA, or store the DNA at -80°C until needed.

RNA Binding

9. Add equal volume of 100% ethanol to each volume of the FT1 (See step 3). Pipette up and down for several times to mix thoroughly, then transfer the mixture to the RNA Spin Column. Centrifuge at 4,000 × g for 1 minute.

RNA Column Washing

10. Add 500 µl Wash Buffer 2 to the RNA Spin Column. Centrifuge at 12,000 × g for 1 minute.
11. Put the collection tube back into the RNA Spin Column, centrifuge at 12,000 × g for 1 minute.
12. Don't need to pour off the liquid, directly transfer the RNA Spin Column to a new RNase-free 1.5 ml centrifuge tube, open the lid and keep in the air for 2 minutes.

RNA Elution

13. Add 30 ~ 50 µl of Elution Buffer to the membrane in the center of the RNA Spin Column, and incubate at room temperature for 2 minutes.
14. Centrifuge at 12,000 × g for 1 minute. 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

Use this product to extract DNA and RNA from 1 × 10^6 293T cells in 2 replicates. Both DNA and RNA elution volumes are 30 µl. The experimental data and agarose gel electrophoresis results are as follows:

DNA	Concentration (ng/ul)	260/280	260/230
1	158	1.85	2.31
2	150.3	1.85	2.11
RNA	**Concentration (ng/ul)**	**260/280**	**260/230**
1	273.5	2.04	2.07
2	278.4	2.05	2.04

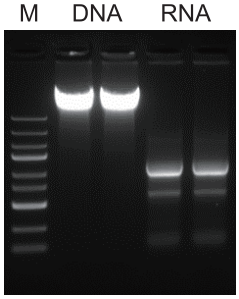


Figure 1. The result showed that, the ***EZ*Bioscience**® EZ-press DNA & RNA Purification Kit can quickly and efficiently extract high-quality DNA and RNA from the same sample.

Trouble shooting

The quantity of DNA & RNA purified by this 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 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 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 DNA and RNA purification by this Kit, **it must be manipulated at room temperature but not on ice** (until the DNA or 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 100% ethanol to the Wash Buffer 2 as indicated on the tag** and mix thoroughly.
3. To get better results, **wash the cells once with PBS** before cell lysing is recommended.
4. **The cells or tissues lysates should be mixed adequately by using vortex**. Then transfer the homogenized lysate of step 1 to the DNA Column and centrifuge.
5. Then **Transfer the RNA containing flow-through FT1 to a new RNase-free 1.5 ml centrifuge tube** for the isolation of RNA. Add equal volume of 100% ethanol to each volume of the FT1 and mix thoroughly, and then add to the RNA column.
6. Optional manipulation: After the procedure of RNA Binding (step 11), add 12 µl diluted gDNA Remover (provided independently) to each RNA Column (2 µl of gDNA Remover diluted with 10 µl ddH_2O), and keep at room temperature for 5 minutes.
7. **DNA should be washed first by Wash buffer 1, then by Wash buffer 2. RNA should be washed just by Wash buffer 2.**
8. When washing the sample, the centrifuge should be set at 12,000 × g, to eliminate the Wash Buffer adequately, and then **open the lid and keep in the air for 2 minutes**.
9. The volume of Elution Buffer can be adjusted between 20 µl and 30 µl, according to the RNA quantity predicted.