EZ-Press 96 RNA Purification Kit

Cat. No.: EZ4001

Description

The EZ-Press 96 RNA Purification Kit is an ideal reagent kit for simultaneously isolating 96 or 192 RNA samples from 2×10^4 to 5×10^5 human or animal cells. The EZ-Press 96 RNA Purification Kit provides an efficient and high-throughput solution for RNA sample preparation in areas such as drug screening, treatment monitoring, and basic research. In less than 1 hour, when processing two 96-well RNA plates in parallel, up to 192 high-purity RNA samples can be obtained (approximately 20 seconds per RNA sample).

The EZ-Press 96 RNA Purification Kit replaces current time-consuming and complex methods, including ethanol precipitation, extensive washing steps, or the use of toxic substances such as phenol and chloroform. The purified RNA can be used for various downstream applications. Including cDNA synthesis, RT-PCR, RT-qPCR, Northern, dot, and slot blot analysis, as well as primer extension, RNase/S1 nuclease protection, and gene chip detection. In addition, the EZ-Press 96 RNA Purification Kit can be used for purifying RNA from enzyme-catalyzed reactions, such as DNA digestion, protein digestion, RNA ligation, labeling reactions, etc.

Components

Cat.No. (Size) / Components	EZ4001-S (2 × 96 Preps)	EZ4001-L (12 × 96 Preps)
96-Well RNA Plates*1	2	12
Caps for Elution Plates*1	2	12
Micro-pore Tape Sheets*1	4	24
96-Well Collection Plates (Square Hole) *1	2	12
96-Well Elution Plates (Round Hole) *1	1 × 2	1 × 12
Buffer RLB	120 ml	240 ml × 3
Buffer RW1*2	70 ml	210 ml × 2
Buffer RW2*2	30 ml	90 ml × 2
Elution Buffer*3	30 ml	100 ml × 2

Note: *1. For the sake of simplicity, these 5 devices will be referred to as RNA extraction well plate, plate lid, breathable sealing film/sealing film, collection well plate, and elution well plate in this article. *2. Before using Buffer RW1 and Buffer RW2 for the first time, it is necessary to add the volume of anhydrous ethanol indicated on the bottle label (the volume ratio of Buffer RW1 to anhydrous ethanol is 1:1, and the volume ratio of Buffer RW2 to anhydrous ethanol is 1:4), and mix thoroughly before use. *3. The Elution Buffer in this reagent kit is Nuclease-free ddH_2O.

Storage

All components should be stored at room temperature and can be stably stored for 12 months. See the expiration date on the product label for details.

Notice

1. Before using Buffer RW1 and Buffer RW2 for the first time, it is necessary to add the volume of anhydrous ethanol indicated on the bottle label (the volume ratio of Buffer RW1 to anhydrous ethanol is 1:1, and the volume ratio of Buffer RW2 to anhydrous ethanol is 1:4), and mix thoroughly before use.
2. Regarding centrifugation: all centrifugation operations with the well plates require placing the 96-Well RNA Plates (hereinafter referred to as "RNA extraction plate") on the 96-Well Collection Plates (hereinafter referred to as "collection plate") or the 96-Well Elution Plates (hereinafter referred to as "elution plate") as needed, after adding the liquid, cover the RNA extraction plate with a Micro-pore Tape Sheet (hereinafter referred to as "breathable sealing film/sealing film") before centrifugation. The collection plate needs to be reused during the cleaning stage, and a separate elution plate should be used during the elution

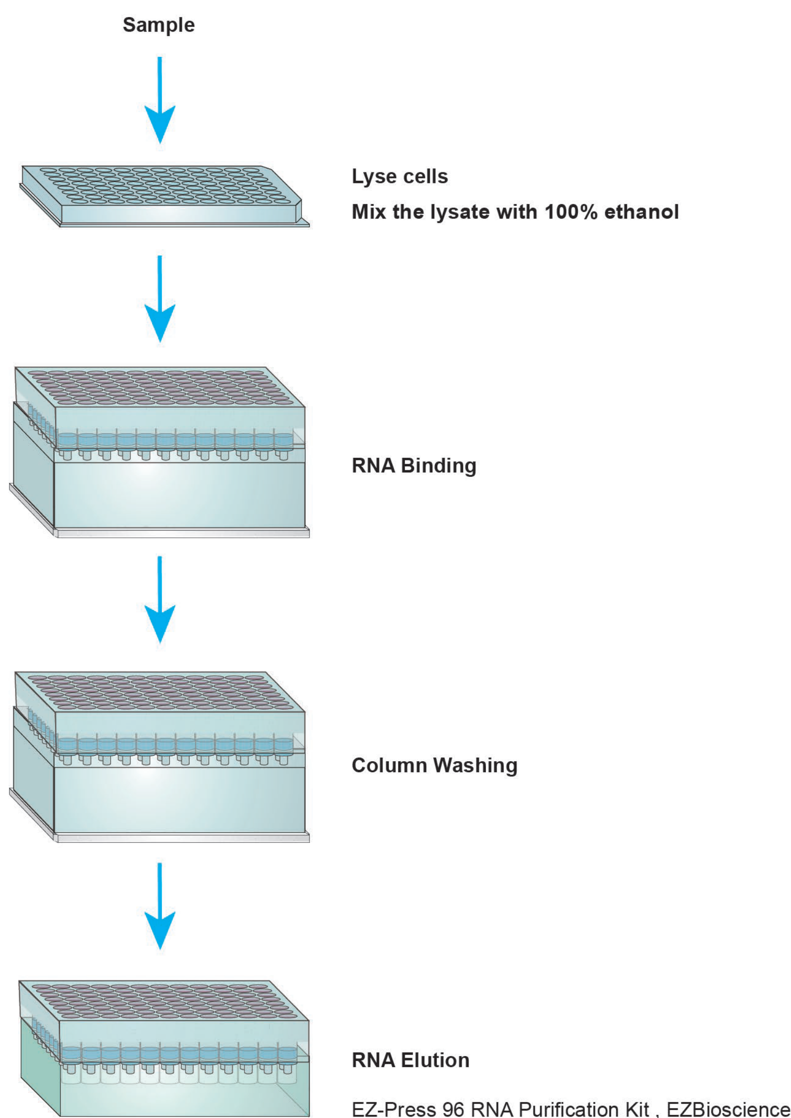
stage.

3. The recommended range for the cell counts of each sample is 2×10^4 to 5×10^5.
4. For your safety and health, please wear a lab coat, disposable latex gloves, and disposable masks.
5. The total RNA extracted by this kit may contain a very small amount of genomic DNA, which will not significantly affect the subsequent reverse transcription and qPCR (especially when the reverse transcription step includes a process to remove genomic DNA, or when the qPCR primers are designed to span intron). For experiments that are highly sensitive to residual genomic DNA, it is recommended to use the EZ4002 kit (which includes a dedicated genomic DNA removal plate that can effectively remove genomic DNA).

Materials

Tabletop centrifuge with swing-out rotor for deep-well plates at a speed of 2100 g or higher, anhydrous ethanol, horizontal shaker, etc.

Experimental Procedure Overview



Protocol

1. Place several collection plates (to be reused) according to the number of cell samples, place the same number of RNA extraction plates on top, and label them properly.
2. Cell washing

a. Adherent cells: After discarding the culture medium, wash the cells once with PBS, then discard the PBS.

b. Floating cells: Centrifuge the cell culture plate at 250 g for 5 minutes, and carefully discard the supernatant.

3. Cell lysis

a. Cells cultured in a 96-well plate: Add 150 μl of Buffer RLB (Lysis Buffer) to each well, and shake horizontally on a shaker at 150 rpm (120 ~ 180 rpm) for 5 minutes.

b. Cells cultured in a 48-well or 24-well plate: Add 150 μl of Buffer RLB (Lysis Buffer) to each well, and shake horizontally on a shaker at 150 rpm (120 ~ 180 rpm) for 5 minutes.

4. Add an equal volume of anhydrous ethanol to each well, and mix by pipetting 10 times. Transfer the mixture to the pre-prepared RNA extraction plate (keeping the same order as the samples in the culture plate). Cover with a breathable sealing film (to prevent impurities from entering the wells during centrifugation).
5. Place the RNA extraction plate on a horizontal centrifuge at 2100 ~ 5000 g for 4 minutes, until the liquid is completely centrifuged down. Then stop the centrifuge, pour out the waste liquid, invert the collection plate onto a clean absorbent paper, and tap it twice to remove any remaining liquid. Then place the RNA extraction plate back onto the same collection plate and tear off the sealing film.
6. Add 600 μl of Buffer RW1, cover it with sealing film, and place it on a horizontal centrifuge at 2100 g ~ 5000 g (the higher the centrifugation speed, the better, and it can be set to the maximum speed of the centrifuge). Centrifuge for 4 minutes until the liquid is completely centrifuged down, then stop the centrifugation. Remove the RNA extraction plate, discard the waste liquid, invert the collection plate onto absorbent paper, and tap it twice to remove the remaining liquid. Then place the RNA extraction plate back onto the same collection plate and tear off the sealing film.
7. Add 600 μl of Buffer RW2, cover with sealing film, and centrifuge horizontally at 2100 g ~ 5000 g (set to the highest speed of the centrifuge) for 12 minutes to thoroughly remove Buffer RW2.
8. Place the centrifuged RNA extraction plate onto a new elution plate, discard the old sealing film, and let it sit at room temperature for 3 minutes to allow ethanol to evaporate completely. Then, add 30 μl of Elution Buffer (or Nuclease-free ddH_2O) onto the membrane in each well, cover it with a new breathable sealing film, and let it sit at room temperature for 1 minute, then place it on a horizontal centrifuge, centrifuge at room temperature at 2100 g ~ 5000 g for 4 minutes, and elute RNA.
9. After centrifugation, remove the above-mentioned RNA extraction plate and elution plate, tear off the sealing film, and add 30 μl of Elution Buffer (or Nuclease-free ddH_2O) onto the membrane in the middle of each well of the RNA extraction plate. Cover with the sealing film (no need to replace the sealing film) and leave at room temperature for 1 minute, then place it on a horizontal centrifuge at 2100 ~ 5000 g for 4 minutes at room temperature to elute RNA (the eluted volume will be approximately 15 μl less than the total volume added).
10. Cover the RNA plate obtained from washing with the matching plate lid. Take 1 ~ 2 μl of RNA and measure the concentration and purity using Nanodrop (when measuring the concentration, use Elution Buffer as a blank control, and if necessary, run a gel to observe the bands. If gel electrophoresis is required, it is recommended to double the amount of Loading Buffer used), Each sample is reverse transcribed with 100 ng to 1 μg of RNA (usually 10 μl of the RNA is used as a template for the reverse transcription reaction). The RNA is then stored at -80°C.

Representative results

The results of the total RNA extracted from a 5×10^4 ~ 2×10^5 cell sample of 293T cells using this reagent kit and detected by Nanodrop are as follows:

Sample	Concentration (ng/μl)	OD260	OD280	260/280	260/230
2×10^5	113.1	2.828	1.385	2.04	2.16
1×10^5	46.2	1.154	0.559	2.07	2.06
5×10^4	18.8	0.469	0.230	2.04	1.81

From the above table, it can be seen that this reagent kit can extract total RNA with relatively high concentration and high purity. When the number of cells is not less than 5×10^4, good OD ratios can be obtained. The total RNA obtained from 5×10^4 293T cells is approximately 600 ng ~ 1 μg.

Trouble Shooting

Questions	Possible causes	Resolution
Low RNA yield	Insufficient lysis or excessive cell volume clogs the RNA adsorption membrane.	Control the number of cells per sample within the recommended range: 2×10^4 ~ 5×10^5.
		Increase the amount of Buffer RLB to ensure adequate cell lysis.
RNA degradation	Improper sample handling	Make sure there are no interruptions during operation. Once the cells have been sufficiently lysed, it is recommended to complete the next steps as soon as possible.
	RNase contamination	Ensure that the reagents and consumables used during the procedure are RNase-free. Before each experiment, the shaft of the centrifuge and the horizontal centrifuge basket must be carefully wiped with a nuclease removal reagent to inactivate the nuclease.
When the number of cells is less than 5×10^4, the A260/280 and A260/230 ratios of RNA are low, which will affect the subsequent experiments.	The low concentration of RNA results in the background absorbance of trace residual impurities affecting the UV absorbance value of the RNA.	It is recommended to increase the cell volume, but the number of residual impurities is minimal and has no effect on subsequent reactions such as reverse transcription and qPCR.