Revision Date: June 2024

EZ-Press 96 RNA Purification Kit PLUS

Cat. No.: EZ4002

Description

The EZ-Press 96 RNA Purification Kit PLUS 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 PLUS 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 PLUS 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 PLUS 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	EZ4002-S (2 × 96 Preps)	EZ4002-L (12 × 96 Preps)
gDNA Removing 96 Plates*1	2	12
96-Well RNA Plates*1	2	12
Micro-pore Tape Sheets*1	6	36
Caps for Elution Plates*1	2	12
96-Well Collection Plates (Square Hole) *1	4	12 × 2
96-Well Elution Plates (Round Hole) *1	2	12
Buffer RLB	40 ml	240 ml
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 6 devices will be referred to as DNA clearance well plate, RNA extraction well plate, breathable sealing film/sealing film, plate lid, 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

The gDNA Removing 96 Plates in this product should be stored at 2 ~ 8°C, while all other components should be stored at room temperature (15 ~ 25°C) 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: During the DNA removal step, it is necessary to place the gDNA Removing 96 Plates (hereinafter referred to as " DNA clearance plate ") on the 96-Well Collection Plates (hereinafter referred to as " collection plate "). After

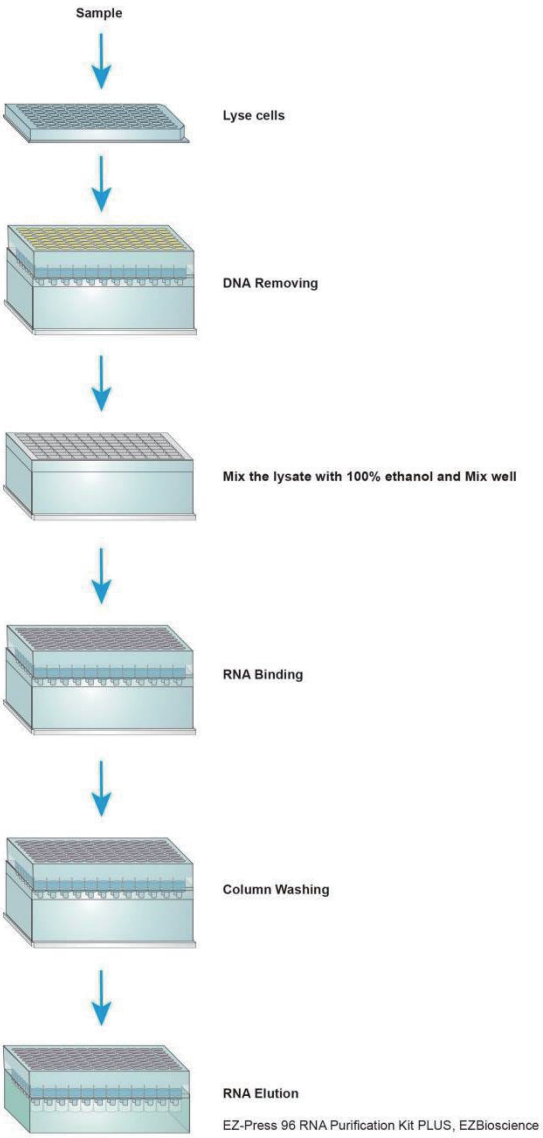
adding the liquid, cover the DNA cleanup plate with a Micro-pore Tape Sheet (hereinafter referred to as "breathable sealing film/sealing film") before centrifugation; during the RNA extraction and purification steps, place the 96-Well RNA Plates (hereinafter referred to as " RNA extraction plate ") on the collection plate. After adding the liquid, cover the RNA extraction plate with a breathable sealing film before centrifugation. During the RNA elution step, the RNA extraction plate needs to be placed on the 96-Well Elution Plates (referred to as the elution plates below). After adding the liquid and before centrifugation, cover the RNA extraction plate with a breathable sealing film. The collection well plate needs to be reused during the cleaning stage, and the 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.

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



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Protocol

1. Prepare the following materials in the same quantity as the samples in advance: assembly components for the DNA clearance plate and collection plate, assembly components for RNA extraction plate and collection plate (to be reused), and label them accordingly.
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 120 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 I Buffer RLB (Lysis Buffer) to each well, and shake horizontally on a shaker at 150 rpm (120 ~ 180 rpm) for 5 minutes.

4. Transfer the above cell lysate products to the DNA clearance plate in order (keeping the same order as the samples in the culture plate), cover it with a breathable sealing film, and place it on a horizontal centrifuge at 2100 g ~ 5000 g for 4 minutes.
5. After centrifugation, discard the DNA clearance plate. Add an equal volume of anhydrous ethanol to the collection plate, mix by tapping 10 times, and transfer to the pre-placed RNA extraction plate (keeping the same order as the samples in the culture plate). Cover with a sealing film.
6. Place the RNA extraction plate on a horizontal centrifuge at 2100 g ~ 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 on a clean absorbent paper, and tap it twice to remove the remaining liquid. Then put the RNA extraction plate back on the same collection plate and tear off the sealing film.
7. 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.
8. 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.
9. 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.
10. 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).
11. 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

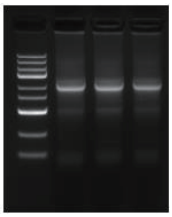
transcription reaction). The RNA is then stored at -80°C.

Representative results

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

Sample	Concentration (ng/μl)	260/280	260/230
1	184.4	2.03	2.00
2	189.7	2.01	2.03
3	177.2	2.02	2.04

The agarose gel electrophoresis results are shown in the figure below. From the gel electrophoresis results, it can be seen that the extracted RNA has high integrity and no obvious genomic DNA residue. This indicates that the total RNA extracted using this reagent kit has high concentration, purity, and integrity, and no genomic residue.



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: $2 \times 10^4 \sim 5 \times 10^5$.
		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 number, but the number of residual impurities is minimal and has no effect on subsequent reactions such as reverse transcription and qPCR.
Gel electrophoresis was performed, and the RNA overflowed into the wells during the loading.	The Loading Buffer concentration is low	Doubling the concentration of the Loading Buffer can be solved.