For research use only Version Number：2.0

Cell Total RNA Isolation Kit

For total RNA purification from cultured cells (10^4 ≤ Cultured Cells ≤ 10^6)

Kit composition	RE-03111	RE-03114
	50 T	200 T
Buffer cRL1*	25 mL	100 mL
Buffer cRL2	15 mL	60 mL
Buffer RW1*	25 mL	100 mL
Buffer RW2	24 mL	96 mL
RNase-Free ddH_2O	10 mL	40 mL
RNA-Only Column	50	200
DNA-Cleaning Column	50	200
Instruction	1	1

*Please wear gloves and take protective measures during the operation as Buffer cRL1 and Buffer RW1 contain irritating chaotropic salts.

Product introduction

This kit adopts centrifugal columns and formulas developed by Foregene, and can extract high purity and quality of total RNA from cultured cells of 96, 24, 12, and 6 well plates with high efficiency. DNA-Cleaning column allows for easy separation of supernatant from lytic cell content and removes genomic DNA. RNA-only Column can efficiently bind to RNA with the help of the uniquely formulated buffer. It is simple and time-saving, while processing a large number of samples.

The entire system is RNase-free. Buffer RW1 and buffer RW2 make the obtained RNA free from contamination by proteins, DNA, ions and organic compounds.

Product information

Format	Spin column	Purification component	Foregene column, reagent
Flux	1-24 samples	Time per prep	~11 min (24 samples)
Centrifuge	Desk centrifuge	Pyrolysis separation	Centrifugal separation
Sample	Cultured cell	Samples amount	10^4 -10^6 cells
Elution volume	20-100 μL	Maximum loading volume	850 μL

Storage

This kit can be stored for 24 months at room temperature (15-25℃) and in dry environment; For further storage, please place it in 2-8℃ environment. Buffer cRL1 can be stored for a month at 4℃ after adding 2-hydroxy-1-ethanethiol (optional).

Note: If stored at a low temperature, it is easy to form the precipitate. Please place the kit solution at room temperature (15-25°C) for a few minutes before use, or preheat it in a 37°C water bath for 10 minutes to dissolve the precipitate before use.

RNA yield and purity

The yield of RNA purified by Cell Total RNA Isolation Kit is related to initial cell amount, freshness, preservation time and operation. The following is the yield and purity of RNA by using the kit to extract RNA from various several cultured cells. In practice, there might be small differences.

Cell Type	RNA yield (μg) / 10^6 cells	OD260/280	OD260/230
MKN-74	15-20	1.8-2.1	1.8-2.1
293T	14-18	1.8-2.1	1.8-2.1
SKBR3	15-20	1.8-2.1	1.8-2.1

Note：(Be sure to read the notes carefully before using)

- All procedures are carried out at room temperature(15-25℃), including centrifugation. **Do not use ice bath or centrifuge at low temperature (4°C).**
- The sample should avoid repeated freezing and thawing, otherwise it will lead to the degradation of the extracted RNA and the yield of RNA will also decrease.
- The yield and quality of RNA is tightly related to sample size and volume of elution. For every 500 μL of buffer cRL1, the recommended maximum cell volume is 10^6.
- Before using the kit, please add 2-hydroxy-1-ethanethiol to Buffer cRL1 (10 μL 2-hydroxy-1-ethanethiol per 1 mL Buffer cRL1). Buffer cRL1 can be stored at 4℃ for 1 month after adding 2-hydroxy-1-ethanethiol. If the extracted RNA is not used to clone full-length cDNA, but only used for other downstream operations such as qPCR or sequencing analysis, it is not necessary to add 2-hydroxy-1-ethanethiol, and the result will not be affected.
- Before using the kit, please add anhydrous ethanol to buffer cRL2 and Buffer RW2 referring to the label on the reagent bottle for the dosage.
- The volume of elution should be no less than 20 μL, otherwise it will influence RNA yield.

◆ Please check if there is any precipitate in Buffer cRL1 and Buffer RW1. If the precipitate can be seen after storage at low temperature, the solution should be placed at room temperature(15-25℃) or 37℃ for a period of time. Use the solution after dissolving and blending.

Procedure (Do Not ice bath or low temperature (4℃) centrifugation)

Before using, add ethanol to Buffer cRL2 and Buffer RW2 referring to the label on the bottle.

1. Please lyse cells according to different types and sources.
   a) Adherent cells: Make sure that the cell culture medium has been completely removed, then use Buffer cRL1 (The amount of addition is shown in the table below) to digest and lyse cells. Or add Buffer cRL1 (The amount of addition is shown in the table below) to the collected cells by centrifugation, and then repeatedly pipette the cells (Until aggregated cells cannot be seen).
   Note: Please double check that there is no cell culture media left, or it may influence RNA yield and purity.
   b) Suspension cells: collect cells by centrifugation, then add Buffer cRL1 (The amount of addition is shown in the table below), pipette the cells repeatedly (Until aggregated cells cannot be seen).
   Note: The RNA in Buffer cRL1 can resist RNase degradation. The cells after being lysed by Buffer cRL1 can be stored at room temperature for approximately 24 hours if they are not used immediately. Also, they can be stored at 4℃ for about 1 week, and for longtime storage, please keep them in -80℃. Heat them in 37℃ or leave them at room temperature before use.

Cell culture vessel	Cell amount	Addition amount reagents (μL)	
		Buffer cRL1	Buffer cRL2
96/48/24/12 well plate	< 10^6	250	400
6 well plate/3.5cm plate	~ 10^6	500	800
≥ 6cm plate	>10^6	Suggestion: use Animal Total RNA Isolation Kit (RE-03011), or take no more than 10^6 cells and use Cell Total RNA Isolation Kit (RE-03111)	
Cultured bottle			

2. Transfer the cell lysate to DNA-Cleaning Column (put DNA-Cleaning Column into the collection tube), and centrifuge at 12,000 rpm (~13,400 ×g) for 2 minutes. Remove DNA-Cleaning Column and keep the supernatant in the collection tube.
   Note: If there is precipitation in the bottom of collection tube, transfer the supernatant to a clean centrifugation tube before performing step 3.
3. Add Buffer cRL2 into the supernatant (Volume of supernatant: Buffer cRL2 = 1:1.6), mixing gently.
   Note: The amount of Buffer cRL2 added depends on the actual volume of supernatant. For instance, add 400 μL Buffer cRL2 to 250 μL supernatant. If there is floc precipitation or the mixture solution becomes turbid, please proceed directly to step 4.
4. Transfer the mixture solution (about 700 μL) to RNA-Only Column (put RNA-Only Column into collection tube), centrifuge at 12,000 rpm (~13,400 ×g) for 1 minute, and discard the effluent in collection tube.
   Note: If RNA is isolated from cells in a 6-well plate or 3.5 cm plate, pass the mixed solution through the column in two batches.
5. Put RNA-Only Column back into the collection tube, transfer all the remaining mixture solution to it, centrifuge at 12,000 rpm (~13,400 ×g) for 1 minute, and discard the effluent in collection tube.
6. Add 500 μL Buffer RW1 to RNA-Only Column, centrifuge at 12,000 rpm (~13,400 ×g) for 1 minute, and discard the effluent in collection tube.
7. Add 700 μL Buffer RW2 to RNA-Only Column, centrifuge at 12,000 rpm (~13,400 ×g) for 1 minute, and discard the effluent in collection tube.
8. Repeat step 7.
9. Put RNA-Only Column back into the collection tube, centrifuge at 12,000 rpm (~13,400 ×g) for 2 minutes to remove the remaining Buffer RW2 in RNA-Only Column.
10. Transfer RNA-Only Column to a new RNase-Free centrifugal tube, add 20-50 uL RNase-Free ddH_2O that has already been heated at 65℃ to the center of the membrane of RNA-Only Column. Leave it at room temperature for 2 minutes, centrifuge at 12,000 rpm (~13,400 ×g) for 1 minute, and then collect the RNA solution.
    Note: The volume of RNase-Free ddH_2O should not be less than 20 uL, otherwise it may affect the elution efficiency. The RNA solution after centrifugation can be added to the membrane of RNA-Only Column again, and step 10 can be repeated to increase RNA yield.
    The product RNA can be used directly in downstream experiment or stored in -80℃. Due to the careful protection of secondary RNA structure supplied by this kit, it is better to leave the product RNA in 72℃ for 5-10 minutes to dissociate the secondary structure before the gel electrophoresis