RG-22

Automated_Total RNA Extraction from Cultured HL-60 Cells (For ~ 1 x 10^6 cells)

Protocol

Pelleted cells

Pellet cells and remove all supernatant by aspiration
(Do not use more than 1×10^6 cells)

← PBS: 20 µl (For frozen cell pellet)

Loosen the pelleted cells by flicking the tube

← LRC (2-ME added) *1 : 520 µl

Transfer all lysate into a 2 ml microtube (sample tube)*2.

On-dish lysis

Remove all medium in the dish by aspiration
(Do not use more than 1×10^6 cells)

← LRC (2-ME added) *1 : 520 µl

Collect lysed cells with a cell scraper into a 2 ml microtube (sample tube)*2

Mix by voltexing mixer at the maximum speed : 1 min

Set into the device

Protocol: RNA CULTURE CELL
(Elution volume : 100 µl *3)

*Please refer to Quick Start Guide or operation manual to know how to set sample tube.

1. Transfer the lysate and mix with 100 µl of Ethanol(>99%).
2. Mix by pipetting.
3. Transfer the lysate and mix with 180 µl of Ethanol(>99%).
4. Mix by pipetting.
5. Apply the lysate into the cartridge
6. Pressurizing
7. Wash 1 time by Wash Buffer (WRC)
8. DNase treatment
9. Wash 2 times by Wash Buffer (WRC)
10. Add selected volume of Elution buffer and elute total RNA into collection tube.

Total RNA

*1 2-Mercaptoethanol (2-ME) must be added to LRC before each use. Add 10 µl of 2-ME per 1 ml of LRC.

*2 Following microtube are recommended.
#BM4020
(BM instrument co., ltd)
#72.695.700,
#72.695.500S
(SARSTEDT)

*3 The default volume of CRC is 100 µl. The volume of CRC can be reduced to 50 µl, but in that case, elution efficiency might be decreased.

Depending on sample and storage conditions, nucleic acid may not be extractable.
Therefore, we cannot guarantee accurate data.
The extracted nucleic acid contains unintended acid (ex: when extracting DNA, RNA is also extracted).

Results

The yield of total RNA (without DNase treatment)
Protein contamination：A260/280 /Chaotropic salt contamination：A260/230

Sample	Number of cells	A260/280	A260/230	Yield (µg)	S. D.
HL-60 cell	1 x 10^6	2.29	2.20	9.8	0.49

N=4

Common protocol is usable for the following

Cultured HeLa Cells, Cultured HEK293 Cells, Cultured NIH/3T3 Cells, Cultured COS-7 cells

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