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Isolation of Total RNA using Fujifilm QuickGene™ RNA Cultured Cell Kit S

To isolate DNA from colonocytes using the Fujifilm Quickgene automated nucleic acid system[1], we recommend using the Fujifilm RNA Cultured Cell Kit S with a modified protocol (a different lysis buffer).

Materials

Fujifilm RNA Cultured Cell Kit S (catalog # RC-S)
Lysis Buffer[2]:100 mM Tris-HCl, pH 7.5
500 mM Lithium Chloride
1 % Lithium dodecyl sulfate
10 mM EDTA, pH 8.0
5 mM dithiothreitol
2-Mercaptoethanol
Ethanol (>99%)
DNase Solution:
2U/µl DNase
10x Reaction Buffer
RNase-Free water

Procedure

1. Begin with 1~2 million cells, based on the 5-8µm count.
2. Centrifuge cells 5min at 900 x g and discard supernatant
3. Resuspend cells in 1 ml PBS. Centrifuge 5 min at 900 x g, discard supernatant.
4. Add Lysis Buffer: 520µl and 2-ME: 5.2µl
5. Mix thoroughly by vortexing for 1 min at maximum speed.
6. Centrifuge at top speed for 5min
7. Transfer the supernatant to 1.5 ml micro centrifuge tube
8. Add >99%Ethanol: 100µl
9. Mix thoroughly by vortexing for 5-15 sec. at maximum speed, flash spin down
9. Add >99%Ethanol: 180µl
10. Mix thoroughly by vortexing for 5-15 sec. at maximum speed, flash spin down
11. Transfer the whole lysate to cartridge in QuickGene system.
12. Select “RNA Cell Plus” mode, press “Start” button
13. After the first washing step, display will show “START SW -> RESTART”
14. Add DNase Solution manually: 40µl

2U/µl DNase	20µl
10x Reaction Buffer	4µl
RNase-Free water	16µl

15. Press Start Button
16 Result: total RNA (Default elution volumn:100µl

[1] http://home.fujifilm.com/products/science/nai_product/product.html
[2] Composition is taken from DYNAL

