# Purification of total RNA (microRNA and mRNA) from liquid samples

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ABSTRACT

Total RNA was extracted from cells and the cultured supernatant and exosome which was extracted from the same amount of supernatant using 3D-Gene® RNA extraction reagent.Comprehensive miRNA and mRNA expression analysis were performed using 3D-Gene® Human miRNA Oligo Chip (miRBase ver.21) and mRNA Oligo Chip(Toray Industries, Inc.) which are featured with the columnar structure and bead-mixing for high sensitivity.

EXTERNAL LINK

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PROTOCOL STATUS

**Working**

1 For 300ul of cultured supernatant and exosome in 1.7ml centrifuge tube,
add 900 µl 3D-Gene RNA extraction (Toray Industries,Inc., Kanagawa, Japan)reagent from a liquid sample kit.

2 Homogenize by vortexing for 1min to mix.
Place the tube containing the homogenate on the benchtop
00:03:00 incubation 25 °C

3 00:03:00 incubation 0 °C

4 Centrifuge for 10 min at 12,000 x g at 4°C without brakes.
After centrifugation, heat the centrifuge up to room temperature.
00:03:00 incubation 25 °C

5 Transfer the 300ul of upper aqueous phase to a new collection tube.

6 Add 450ul of 100% ethanol and mix thoroughly by pipetting up and down several times. Do not centrifuge.

7 Transfer the 750ul of sample into the silica-based membrane column and purification according to general nucleic acid purification protocol.

8 Elute the RNA with RNase free water, and concentrate to 4ul.

9 Comprehensive miRNA and mRNA expression analysis were performed using 3D-Gene® Human miRNA Oligo Chip (miRBase ver.21) and mRNA Oligo Chip(Toray Industries, Inc.) which are featured with the columnar structure and bead-mixing for high sensitivity.