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

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Atsuko Mizoguchi¹

¹toray industries Inc.,

Atsuko Mizoguchi

Toray Industries, Inc

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.

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- 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 1 min 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.