Research Center for
Pharmaceutical Nanotechnology
Tabriz University of Medical Sciences
RCPN

microRNA extraction protocol

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RCPN

Detailed procedure

microRNA extraction protocol

Step1. Total RNA extraction

1. Add 1 mL Trizol to the homogenized cell pellets or body fluids (200 µL) and mix well by vortexing and incubate at room temperature for 10 min.
2. Add chloroform (0.2 v/v) to separate the aqueous and the organic phase and mix by inverting the tubes for 10 sec. Incubate the samples 5 min at room temperature for the complete dissociation of nucleoprotein complexes.

   Note: do not vortex samples in this step.
3. Centrifuged the sample at 12,000 ×g for 12 min at 4 °C. All RNAs, including miRNAs, are in the aqueous phase, which is the top clear phase.

Step2. Precipitation of large RNAs and enrichment of small RNAs

1. Add 3 M sodium acetate, pH 5.2 (1/10 v/v) and incubate in -20°C for 30 min.
2. Centrifuged the tubes at 12,000 ×g for 12 min.
3. Transfer the supernatant into a new tube and add equal volume of 2.5 M LiCl (v/v) and 2 volumes of precooled absolute ethanol (v/v).
4. Incubate the samples at -80 °C.
5. Centrifuge the tube at high speed 16,000 ×g, 4 °C for 20 min.
6. Dry the pellet and dissolve it in 20µL DEPC water (65 °C, 5 min).

Note: water solubility of the pellet may be decreased if it excessively dried.

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Reference

For more information read “A microRNA isolation method from clinical samples” article *(BioImpacts*, 2016, 6(1), 25-31.doi: 10.15171/bi.2016.04).

Good luck!

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