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RNA extraction_Trizol method_Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.98uh9ww

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ABSTRACT

The study goal is to identify the gene expression profile of interscapular brown fat (iBAT)-related ganglia (SG/T1 & T3) and inguinal white fat (Iwat)-related ganglia (T13/L1 & L2)

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MATERIALS TEXT

- Trizol: Ambion® by Life Technologies, Catalog number: 15596-026
- Hand-held homogenizer: PELLET PESTLE® Cordless Motor, motor cordless, kon 749540-0000, kimble pellet pestle motor, kimble chase company.
- Sterile pellet pestles: part number: 749520-0500, kimble chase company.
- Glycoblue: GlycoBlue™, Ambion® by Life Technologies
- Phase Lock Gel™ (PLG): QuantaBio
- Phase separation reagent (BCP, molecular biology grade 1-bromo-3-chloropropane): catalog number: BP 151. Molecular Research Center, Inc.
- 100% isopropanol
- 75% RNase-free ethanol
- Heat block
- RNase free water
- Sterile RNase-free polypropylene micro-centrifuge tubes.
- Micro centrifuge machine
- Vortex mixer
- NanoDrop machine

1 Take out the samples from the -80 °C freezer and keep them stay in the ice.

2 Add 500 µl Trizol (Ambion® by Life Technologies, Catalog number: 15596-026) to each sample (when adding the Trizol, add it toward the tissue so as to make sure the tissue is in the Trizol solution, instead of sticking on the tube well).

3 Use a hand-held homogenizer (PELLET PESTLE® Cordless Motor, motor cordless, kon 749540-0000, kimble pellet pestle motor, kimble chase company) with the sterilized (by autoclaving) pellet pestles **(**part number: 749520-0500, kimble chase company) to homogenize the samples in the fume hood (Use the pellet to locate the tissue in between the micro-centrifuge tube well and the tip and squeeze it, then turn on the homogenizer in the tube).

3.1 Note: Change the homogenizer tip for each sample. After it's done, check the solution again to make sure the tissue is completely homogenized.

4 Add 1.7 µL Glycoblue (GlycoBlue^{TM}, Ambion® by Life Technologies)

Note: 300-fold dilution; the total volume of the solution for each sample is 500 µL, so after diluting 300-fold dilution, the needed volume of glycoblue is 500/300=1.7 µL to each sample.

5 Centrifuge 30 phase lock gel (PLG) at the maximum speed of the centrifuge machine for 1 minute.

6 Transfer homogenized samples to the PLG micro-centrifuge tubes and incubate the homogenized sample at room temperature for 5 minutes.

7 Add 100 µl phase separation reagent (BCP, molecular biology grade 1-bromo-3-chloropropane) (catalog number: BP 151), Shake vigorously by hand for 15 seconds (by putting another micro-centrifuge tube rack on top of the micro-centrifuge tube rack that contains these sample tubes, and then shake them). Incubate at room temperature for 2-3 minutes.

8 Then centrifuge the sample at 12,000×g for 15 minutes at 4 °C.

9 Transfer the upper aqueous phase into new RNase-free polypropylene micro-centrifuge tubes.

10 Add 0.25 ml 100% isopropanol to the aqueous phase, mix well and incubate at room temperature for 10 minutes.

11 Centrifuge at 12,000 ×g at 4 °C for 15 min.

12 Remove the supernatant and wash the pellet with 500 µl 75% RNase-free ethanol.

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13 Vortex the sample briefly and centrifuge at 7500 ×g at at 4 °C for 5 minutes.

14 Discard the ethanol and dry the RNA pellet in the heat block at 55-60 °C (avoid the RNA from being over dried).

15 Resuspend the RNA pellet in 20 µl RNase free water and incubate in a heat block set at 55-60 °C for 10-55 minutes.

16 Measure RNA concentrations with NanoDrop and proceed to the downstream application, or store at -80°C.

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