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SOP for TriZOL RNA and Protein Extraction

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We use this protocol and it's working

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Abstract

SOP for TriZOL RNA and Protein Extraction

Prepration

1

1.1 Bucket of ice and dry ice

1.2 RNAse Zap work surface and hands prior to start

1.3 Per sample: 2 1.5 mL clear tubes, 1 RNeasy tube (pink), 1 QIAshredder tube (purple)

1.4 Label all tubes (clear tubes very specific) and set up in order of clear, purple pink, clear.

1.5 Keep all samples on dry ice before TriZOL is added.

Lyse Tissue

2 In hood, add (500uL or 1000uL depending on tissue size) ice cold TRIzol to 2mL tube containing sample and 1 tissue lyser bead.

3 Place in Tissue lyser adaptors. Tubes in on “big side” of adaptors, “big side” facing out when lysing. Operate tissue lyser for 2min at 20Hz. Rearrange racks. Repeat cycle 2-3x.

4 Quick spin (few seconds) in 4 deg C centrifuge or table top mini centrifuge (to get rid of foam, may not be necessary).

5 Transfer to new 1.5mL tube.

6 Add 1/5 volume chloroform. Shake 15 sec. (invert a few times with hand).

7 Incubate at RT 2-3min.

8 Centrifuge 15min, 12000xrpm, 4 deg C. Layers should separate into clear RNA (top), white DNA (middle), pink protein (bottom). After 15 min, fast temp centrifuge to RT.

9 Transfer upper aqueous phase to either Qiashredder tube for RNA isolation or 1.5mL tube for storage. Keep track of how much RNA phase is transferred, will need to add equal volume of 70% EtOHin step 11. If isolating RNA, proceed to step 11.

10 If isolating DNA or protein place the tube containing lower TRIzol layer on ice until RNA processing is complete. Make sure tubes are upright and still so DNA/protein layers do not mix. Isolate RNA

Isolate RNA

11 Centrifuge Qiashredder 2min, full speed, RT (21-23°C). Retain flow-through; discard column. (Can be stored at -80 deg C for later extraction.)

12 Add 1 volume 70% EtOH to flow-through. Pipette to mix.

13 Transfer up to 700uL of the sample, including any precipitate that may have formed, to an RNeasy spin column in a collection tube. Centrifuge 15sec at 10,000 RPM. Discard flow-through. (If volume is over 700uL, repeat this step until whole volume is used).

14 Add 700uL Buffer RW1 to RNeasy spin column. Centrifuge 15sec at 10,000 RPM. Discard flow-through.

15 Add 500uL Buffer RPE (EtOH added) to RNeasy spin column. Centrifuge 15sec at 10,000 RPM. Discard flow-through.

16 Add 500uL Buffer RPE (EtOH added) to RNeasy spin column. Centrifuge 2min at 10,000 RPM. Discard flow-through.

17 Place RNeasy spin column in new collection tube. Centrifuge 1min at full speed. Discard any flow-through.

18 Place the RNeasy spin column in new 1.5mL Eppendorf tube (the final tube clearly labeled RNA). Add 30-100uL RNAse-free water directly to the spin column membrane (directly to “white part” of column). Centrifuge 1min at 10,000 RPM to elute RNA. Put tubes with eluted RNA on ice. Note: with gut samples, start with 50uL RNAse-free water and see what protein conc. Is obtained. If extremely large (over 2000), inc. RNAse-free water volume to 100uL.

19 Measure RNA concentration (comp by JJ desk). Open Nanodrop 2000 → click nucleic acid → “No” to save last workbook → “ok” to wavelength verification → change type to RNA → to blank, add 1uL of RNA water on top of silver head →hit “blank” in top L corner → “measure” icon will become green → click “measure” for each sample. Any conc. above 100ng/uL is usable, but aim for ~1000ng/uL. Goal of 1.9-2.2 for 260/280 and 260/230. Collect Protein

Collect Protein

20 Remove white DNA layer (if you do not remove this entire layer you will have a very hard time re-suspending your protein in SDS)

21 add 1-1.2mL cold MeOH to phenol/chloroform layer; invert to mix

22 Incubate at RT 10min.

23 Centrifuge 10min, 12,000xrpm, 4 deg C.

24 Remove supernatant.

25 Wash pellet with 500uL cold MeOH (pipette up and down with a p1000 tip to mix).

26 Centrifuge 10min, 12,000xrpm, 4 deg C.

27 Remove supernatant, make sure most of the MeOH has evaporated

28 Resuspend pellet in 50-150uL 1% SDS using pestle motor mixer. If using many samples, can rinse mixer in 70%EtOH →water → kim wipe and re use.

29 Heat sample to 50 deg C to resuspend (use p1000). Measure protein concentration by BCA assay.