Icahn School of Medicine at Mount Sinai LINCS Center for Drug Toxicity Signatures

Standard Operating Procedure:
Preparing Proteomics Samples from Trizol Lysis—Protein Extraction and Precipitation

DToxS SOP Index: #A-2.0
Last Revision: 12/29/2015
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Quality Control (QC) steps are indicated with green highlight.

Metadata recording is highlighted with yellow highlight and superscript indices.

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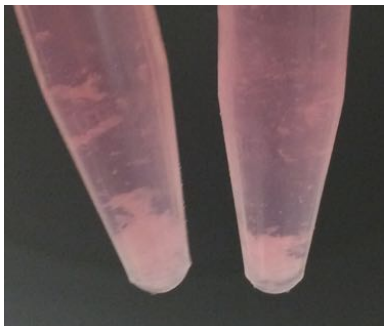
1. Obtain the protein fraction from Trizol lysis of cultured cells. This is defined as what is left after taking the RNA fraction (see SOP A-1.0).
    a. In our case of PromoCell experiments, we have two 1.5 mL tubes (Eppendorf Cat 022431081), each containing ~ 600 µL of of Trizol, Choloroform and MeOH mixture.
2. Add 900 µL of ice-cold acetone (Fisher Chemical Cat A18-4) to each tube using a 1000 µL tip and a 1000 µL micropipette, mixing carefully by repeated gentle pipetting.
    a. There are now two tubes with 1.5 mL of sample each.
3. Take 500 µL of the mixture from each tube, and combine into a new 1.5 mL tube. This will yield a total of three tubes, each with 1 mL of sample.
4. Add 500 µL of ice-cold acetone to each of the three tubes and mix as above.
5. Store at -20^{o}C overnight (at least 10 hours).
6. Centrifuge the tubes at 4^{o}C and 16,000 rpm (23,469g) for 30 min.
    a. We use a Beckman Coulter Allegra 64R centrifuge.
    b. QA/QC1. The final protein pellet color should become progressively whiter, from a reddish/yellowish tint, after each of the wash steps below are completed.
7. Using a 200 µL tip and a 200 µL micropipette, carefully remove 200 µLof the supernatant, repeating until the supernatant is removed completely from all three tubes.
    a. Be very careful not to disturb the pellet.
8. Add 1 mL ice-cold acetone to each tube.
9. Sonicate on ice to break the protein precipitate pellets, then store at -20^{o}C overnight.
    a. We use a Model Branson Sonifier 450, 5 bursts at level 3, with ~1-2 sec between bursts

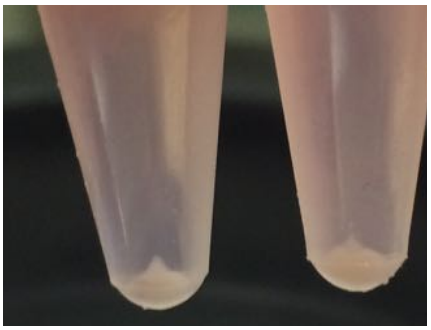
    b. Settings are as follows: Timer: Hold, Duty cycle: 80, Output control: 3.
10. Centrifuge again as in Step 6, and remove supernatant acetone as in Step 7.
11. Repeat Steps 6-8 one more time.
12. Repeat Steps 6-7, but now after removing the supernatant acetone, let the tubes stand open at room temperature for 10-15 min. This allows the remaining acetone to evaporate.
13. Resolubilize the pellets by adding 70 µL of 8M urea (Fisher Chemical Cat U15500) in 50 mM ammonium bicarbonate (Fluka Sigma Aldrich Cat 09830-500G) to each tube..
    a. Vortex as needed to facilitate resolubilization.
14. Store samples at -80^{o}C.

Quality Assurance/Control Steps (QA/QC)

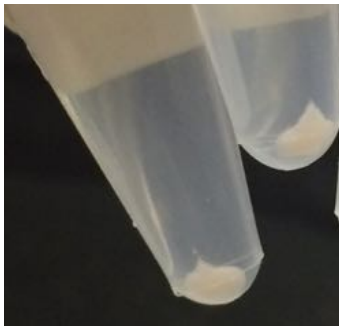
QA/QC1. Protein Pellet Appearance. The protein precipitate from acetone washing at the various stages of the SOP. After spinning, the pellet gets increasingly whiter with more washes.



Before First Spin (Step 5)



After First Spin (Step 6)



After Last Spin (Step 11)