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TCA/DOC protein precipitation protocol

Guidelines for sample preparation
(How to protect your samples from contamination with keratin)

- **TRY TO AVOID ANY CONTACT OF SAMPLES AND SOLUTIONS WITH DUST, SKIN OR HAIR**
- **CLEAN YOUR BENCH**
- **WEAR GLOVES AT ALL TIMES**
- **ALL REAGENTS SHOULD BE PREPARED FRESH**
- **USE ULTRA PURE WATER FOR ALL SOLUTIONS**

This protocol uses TCA in the presence of DOC, suitable for the sample with very low protein amounts (< 1μg) in a large volume (up to 3ml solution). Sometimes it may need to readjust pH after resuspension of the TCA pellet to make it ideal for next step assay.

1. Bring 1.0 ml sample in a 1.5 ml centrifuge tube.
2. Add 8.5 μl of 2 % Na-deoxycholate (DOC), final concentration of 125 μg/ml
3. Vortex
4. Leave at RT for 15 min, add 333 μl 24 % trichloroacetic acid (TCA final concentration 6 %)
5. Vortex
6. Centrifuge 12000 g, 30 min at 4 °C
7. Aspirate supernatant carefully without touch the pellelt
8. Wash once by centrifugation with acetone (-20 °C) to remove excess of TCA
9. Centrifuge 12000 g for 5 min at 4 °C
10. Solubilise pellets in 6 M urea buffer (pH 7.8) for in solution digestion or gel loading buffer for SDS-PAGE

Reference: Bensadoun & Weinstein. 1976. Assays of proteins in the presence of interfering materials. Anal. Biochem. 70:241:250.