Tissue Homogenization

Equipment and Materials:

Reagents:

- T-PER® Tissue protein Extraction Reagent. (*PIERCE*. Product No 78510)
- Complete Mini Protease Inhibitor Cocktail Tablets, (*Roche*, Cat. No 04 693 124 001)

Instrument and Supplies:

- Pestle and tube homogenizer (also called tissue grinder) DUALL®, All-Glass**.** *Kontes.*
    - Choose the homogenizer article according to the volume of medium required, e.g. the article No. 885450-0022 can be used to grind tissue in 5 ml of lysis buffer.
- *Falcon* tubes: BD Falcon #352097, polypropylene (Fisher Cat. #14-959-70C).
- *Eppendorf* tubes: Safe-Lock microcentrifuge tube, 1.5 ml Cat. No 022363204.
- *Fisherbrand* PCR Tube with Attached Cap, PP, 0.2 ml, Flat Cap, Natural. Cat No 08-408-214.
- Eppendorf Centrifuge 5415 C.
- Ultrasonic - Homogenizer – Branson Sonifier 450 Analog.

Procedure:

Note: This procedure describes a protocol for ~250 mg of tissue. It may be necessary to prepare a greater or a smaller volume of solution depending on the amount of starting material, and if a more concentrated protein extract is required. Use a ratio of approximately ~1 g of tissue per 20 ml T-PER Reagent (T-PER manufacturer).

Protease inhibitors Solution Preparation

Note: In general, addition of specific protease inhibitors or cocktails with a broader activity spectrum is recommended during cell disruption and subsequent preparation, to inhibit protease activity. Protease inhibitors should be applied with caution, as it was reported that they may modify proteins, introduce charge trains and adducts, and hence interfere with further studies.

Add 1 tablet of complete Mini Protease Inhibitor Cocktail in 10 ml of T-PER reagent and dissolve thoroughly (manually or by vortexing). If very high proteolytic activity is present, one tablet should be used for 7 ml extraction buffer. Keep the solution on ice.

Tissue Homogenization

1. Remove the tissue from the -80°C freezer and immediately place in dry ice.
2. Cut a piece of tissue using razor blade in the cryostat and thaw on ice.
3. Weigh the piece of the tissue and then place the sample on ice again to keep it from getting warm.
4. Working on ice, chop the sample tissue into small pieces (~1 mm in cross section) with scissors or a single-edge razor blade. It is imperative that the tissues stay cold to minimize protease activity.
5. Place the tissue pieces in the glass tube homogenizer and add an appropriate amount of buffer solution, (or T-PER containing Protease Inhibitor cocktail) according to section 5.0. If 250 mg of tissue is used, add 5 ml of T-PER.
6. Homogenize on ice by grinding with a pestle for several minutes until no more chunks are visible. The pestle is manually worked to the bottom of the tube, thus tearing and fragmenting tissue as it is forced between the sides of the pestle and the wall of the tube.
7. Transfer the ground material to 15 ml Falcon tubes (2.5 ml per tube) and keep them on ice.
8. Sonicate on ice for 5-10 cycles until foaming occurred. Use the Branson Sonifier 450 apparatus with 30% duty cycle and output control set to 3. Turn the knob to a setting >"3" to start and back to "0" to stop.
9. Incubate on ice for 10 min to allow for cell lysis.
10. Transfer the sample in aliquots to 1.5ml Eppendorf tubes and centrifuge in the cold room at 14,000 x g for 10 min, to pellet cell/tissue debris. Carefully collect the supernatant and aliquot ~ 200 uL into a PCR tube on ice.

Note: As a result, a clear supernatant should be obtained. Sometimes excess tissue is transferred over into the PCR tube. If this is the case, spin a second time and transfer the supernatant in a second fresh PCR tube. Store samples in the -80°C until use or continue with downstream analysis for further purification.

Cleaning of tissue grinders.

1. Wash the pestle and tube homogenizer with ethanol followed by a dilute solution of liquid detergent to remove traces of sample.

2. Rinse thoroughly with warm water, then de-ionized water, and finally with Milli-Q water.
3. Proceed to the homogenization of the next tissue if you have more sample tissue. If not, rinse the grinder with methanol and allow to dry.

Expected Outcome:

Upon completion of these steps you should have a protein extract solution amenable to any further biochemical analysis with minimal clean-up.

Note: No single method can be applied to all samples, and there is always the necessity to optimize the procedure for particular samples. All procedures should be as simple as possible and, even more importantly, to be reproducible to avoid protein loss, degradation and modifications.

References:

1. Bodzon-Kulakowska A, Bierczynska-Krzysik A, Dylag T, Drabik A, Suder P, Noga M, Jarzebinska J, Silberring J. Methods for samples preparation in proteomic research. J Chromatogr B Analyt Technol Biomed Life Sci. 2007 Apr 15;849(1-2):1-31. Epub 2006 Nov 20. Review.
2. T-PER® Tissue protein Extraction Reagent, Pierce instructions.
3. Complete Mini, Protease Inhibitor Cocktail Tablets, Roche instructions.