Tissue Lysate preparation - general protocol

Datasheets that this protocol applies to – All those where western blots using goat polyclonals on tissue lysates (human, rat or mouse) are described, except where the data is clearly attributed to an external collaborator.

Starting material: Tissues stored as chucks under liquid nitrogen.

During handling the material is kept on ice. Before generating a lysate, the tissue is first cut into ~1mm cubes by using a razor blade on a glass plate held on ice.

The small cubes are then transferred into a hand held potter homogenizer with three ml ice-cold RIPA buffer per gram of tissue.

- Tough tissues like Prostate, Skin and Thyroid need incubated in the RIPA for 20 in on ice before starting homogenizing
- Medium tough tissues like Colon, Duodenum, Heart, Kidney, Skeletal Muscle or Tonsil, are kept in RIPA on ice for 10min before homogenizing
- Homogenize by pushing the piston slowly into the mix by continuously twisting the wrist thus turning the piston around its axe.
- Make sure all tissue chunks slide between the piston and the glass wall while homogenizing.
- Once the piston reaches the bottom, reverse the handling
- Keep the tissue submerged in the ice during the process
- Repeat the cycle until the tissue is liquefied
- Divide the liquefied tissues over 1.5ml tubes and centrifuge at 13,000rpm for 3 min at 4C
- Transfer the clear supernatant in new clearly labelled tubes. Take 20ul out for protein determination
- Protein determination is carried out using the BioRad Protein Determination Kit.
- Adjust the lysate to 5mg/ml by adding ice cold RIPA buffer
- Store in liquid nitrogen.

RIPA buffer: 20mM Tris-HCL pH7.4, 150mM Na\Cl, 1mM EDTA, 1% Triton-X100, 1% sodium deoxycholate, 0.1% SDS with freshly added PMSF to 1mM and with freshly added aprotinin and leupeptin to 5ug/ml just before use.

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