Tissue Lysate preparation - general protocol

Datasheets that this protocol applies to – All those where western blots using goat polyclonal antibodies 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 min 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.