Proteome Mapping/iTRAQ - General Lab Information © 2011

Protein Extraction from cells/tissue

Examples:

- Cell cultures:
  - Lysis Buffer 1: 0.2% NP40, 40 Mm KCl, 10 mM Hepes, + protease inhibitors OR
  - Lysis Buffer 2: Prepare in PBS, 0.2% IGEPAL, 0.2% Triton X, 0.2% w/v CHAPS, 75 mM NaCl, 1 mM EDTA , protease inhibitors;
  - Centrifuge at 13,000g for 10 min at 4°C, retain supernatant and freeze at -80°C.
- Fungus
  - Freeze dried, mechanically broken with mortar and pestle;
  - Solubilise proteins with 10mM Tris-Cl (pH 7.5);
  - Centrifuged at 20 000g for 15 min @ 4° C;
  - Supernatant treated with nucleases to remove nucleic acids;
  - Retain supernatant and freeze at -80°C.
- Muscle tissue
  - Lysis buffer - 50 mM Tris (pH 7), 0.5 mM EDTA, 20% glycerol, + protease inhibitors;
  - Sonicate sample on ice twice for 10 s with a 5 s delay between bursts;
  - Centrifuge sample at 13000*g* for 10 min at 4 °C;
  - Retain supernatant and freeze at -80°C.

Protein Extraction from Secreted Protein

- Secreted proteins should be in serum/plasma free media for 24 hours prior to harvesting.

Protein Quantity

- iTRAQ requires 25 µg total protein per tag (for a 4 tag experiment);
- To ensure sufficient protein is available after clean-up ~250 µg protein per sample is required for labeling for 2 tag, ~150 µg per sample is adequate for 4 tag. The minimum specifications are because up to 80% of sample can be lost during sample cleanup. Greater amounts of total protein will improve sensitivity towards low abundance proteins; lower quantities may provide sub-optimal results;
- Freeze sample at -80°C.

Notes

- Protease inhibitors can be used to minimise protein degradation;
- PI prefers samples to be delivered in lysis solution on dry ice;
- iTRAQ analysis cannot be used on samples from an unknown genome. Indicate available database on worksheet;
- Indicate on worksheet exactly what solution/buffer used;
- Substances that interfere with process
  - sucrose
  - urea
- Lysis buffers used for 2D gel preps are unsuitable for iTRAQ as they contain substances which interfere with the process.

Transportation of samples

Ideally samples should be dispatched on dry ice to arrive frozen. Alternatives may be possible, contact info@proteomics.com.au with specific details of your situation.