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Proteome Mapping/iTRAQ - General Lab Information © 2016

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.
  - Acetone precipitate the supernatant and freeze at -20°C.
- Fungus
  - Freeze dried, mechanically broken with mortar and pestle;
  - Solubilise proteins with 10 mM Tris-Cl (pH 7.5);
  - Centrifuged at 20,000g for 15 min @ 4°C;
  - Supernatant treated with nucleases to remove nucleic acids;
  - Retain supernatant.
  - Acetone precipitate the supernatant and freeze at -20°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 sec with a 5 sec delay between bursts;
  - Centrifuge sample at 13,000g for 10 min at 4°C; retain supernatant.
  - Acetone precipitate the supernatant and freeze at -20°C.

Protein Extraction from Secreted Protein

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

Protein Quantity

- iTRAQ (4-plex) requires 25 µg to 100 µg total protein per label (for a 4 label experiment).
- iTRAQ (8-plex) requires 20 µg to 100 µg total protein per label (for a 8 label experiment.
- To ensure sufficient protein is available after clean-up (acetone precipitation):
  ~250 µg protein per sample is required for labeling with 2 labels
  ~150 µg protein per sample is required for labeling with 4 labels
  ~100 µg protein is required for labeling with 8 labels
  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.
- Samples should contain a minimum of 100 µg of protein but not exceed 200 µg per tube (except for 2 labels which requires 250 µg).
- Each sample should contain the same amount of starting material (eg.100 mg of leaf, 20 mg of muscle, etc. should be used for all the samples).

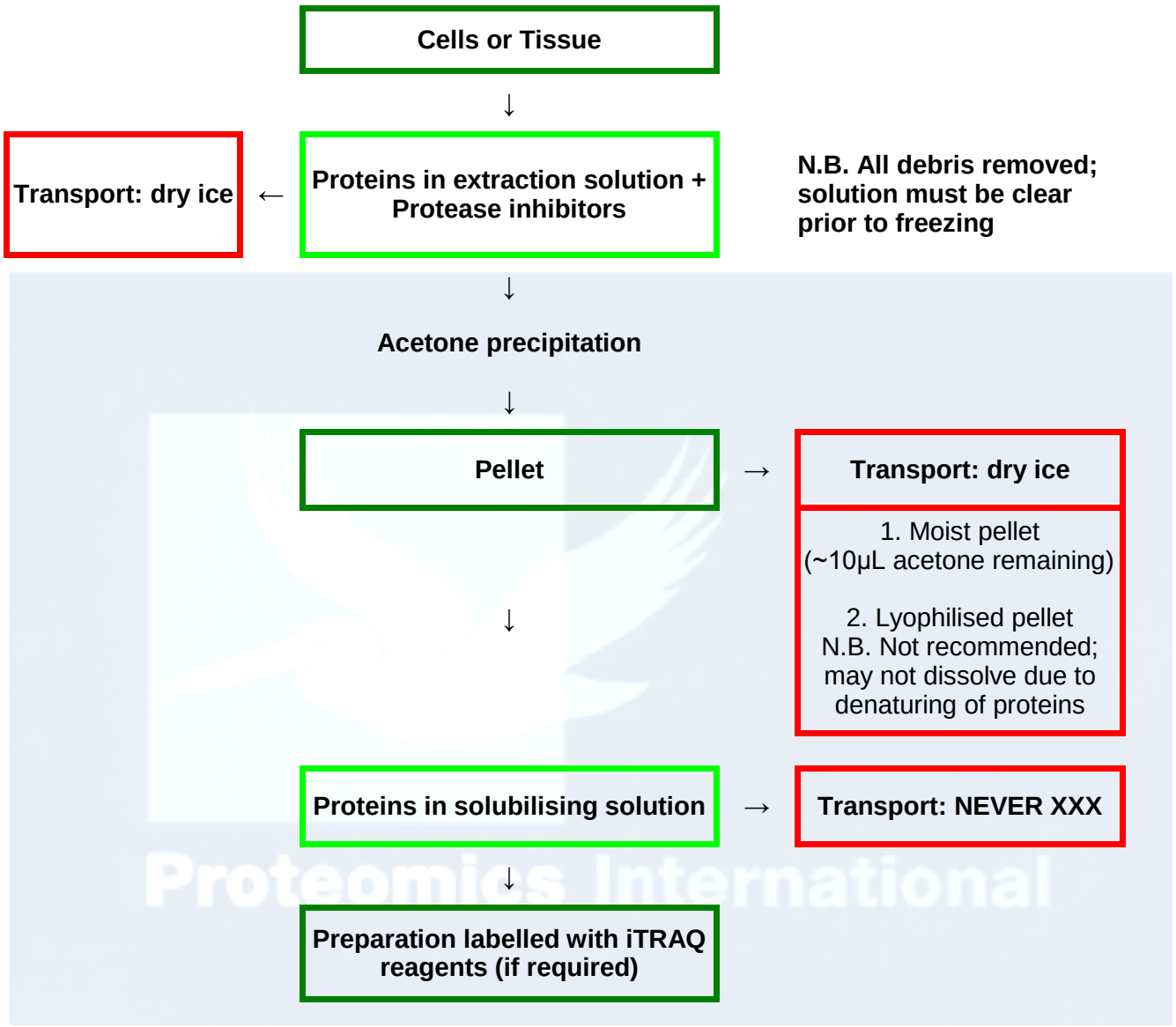
Notes:

- PI prefers samples to be delivered as protein precipitated pellet on ice (transport 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. Alternatives may be possible, contact info@proteomics.com.au with specific details of your situation.

Work flow: Sample Preparation and Shipment for Proteome Mapping/iTRAQ



iTRAQ sample experimental design and questions answered

iTRAQ experiment: 4-plex

Example experiment

Sample name	Control	Sample 1	Sample 2	Sample 3
iTRAQ Label	114	115	116	117

Questions addressed:

1. **Possibility of carrying out biological/technical replicate analyses.**
2. **Results highlight up- and down-regulated proteins across samples for differential expression analyses.**
3. **4-plex experiments provide better proteome coverage (except when using plasma samples).**

iTRAQ experiment: 8-plex

Example experiment

Sample name	Control	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
iTRAQ Label	113	114	115	116	117	118	119	121

Questions addressed:

1. **Possibility of carrying out biological/technical replicate analyses.**
2. **Results highlight up- and down-regulated proteins across samples for differential expression analyses.**
3. **Eight lanes permit the analyses of more samples than a 4-plex experiment.**