Preparation of total protein extracts from cells or organs

Remove tissue as quickly as possible after killing the animal use straight away (cells: wash twice with PBS, scrape off and freeze as before). Determine weight and use appr. 1 ml buffer per 100 mg of tissue.

Homogenize frozen material with Polytron for 2 x 1 min in the following buffer (pre-heated at 95 °C):

50 mM sodium phosphate pH 6,8
5 % SDS
40 mM DTT
5 mM EDTA
5 mM EGTA
20 % Glycerol
0.01 % Bromophenolblue

After homogenization heat 5 min at 98 °C. If sample is still viscous, heat for another 5-10 min. Spin 5 min full speed (benchtop), transfer supernatant in fresh tube. Save pellet just in case.

Use 1-10 µl per gel lane and top up with sample buffer. If 2D electrophoresis is desired precipitate total protein with acetone as described in protocol and resuspend in IEF or NepHGE sample buffer.

Further considerations:

It might be necessary to add protease inhibitors, eg a cocktail of 2 mM PMSF, 0,5 mM E-64 and 100 µg/ml ovomucoid. Other inhibitors are available from Pierce or Boehringer.

Some proteins require a different pH in the sample buffer or might be susceptible to boiling. Here heating to 50-65°C might be sufficient. In this case residual genomic DNA must be sheared by pressing the sample through a fine (27 G) syringe needle.

It is essential that the buffer is either prepared fresh or stored in small aliquots at -20°C to preserve DTT. If samples are stored for long-term, re-add DTT to 20 mM before heating.