2D gel electrophoresis

This protocol is based on GE Lifesciences manual:

https://www.gelifesciences.com/gehcls_images/GELS/Related%20Content/Files/1314716762536/litdoc28953755_20161013182324.pdf

Consumables required for IEF:

Immobiline Drystrip gels
DeStreak Rehydration Solution
IPG buffer

Choose Iso-Electrical Focussing strip: pH range and size

Immobiline DryStrip gels (IPG strips) from GE Healthcare Life Sciences are available in different pH ranges and sizes (see catalogue).

· For a mini-gel use a 70mm strip
· Two 11 cm strips can be easily run in one high resolution gel
· Two 13 cm strips can run, but the acidic end will hang out of the gel

Preparation of protein samples

Proteins from extract, lysate, or from a specific purification procedure should be resuspended in 125 ul of Rehydration Solution for a 70mm strip, not exceeding protein concentration of 1mg/ml.

Add the corresponding IPG buffer (pH range) just before use depending on the equipment used (see manual).
In case of pellets containing Agarose or Sepharose beads, resuspend in 140ul, mix well, spin to pellet the beads and pipet out 125ul (for 7cm) 250ul (for 13cm) for strip rehydration.

Rehydration of the Dry Strip

· Add 125ul of the protein sample/rehydration buffer to the groove in the rehydration tray
· Peel off the cover foil from the strips with forceps
· Place the strips on top of the protein sample (gel side down). Remove any air bubbles by sliding strip carefully into the groove
· Cover the strips with mineral oil to avoid evaporation and crystallization of urea
· Rehydrate strip for a minimum of 12 hours (overnight)

Separation of the first dimension
· Remove the strip from the rehydration tray and place it in the flat bed of the Isoelectric Focusing Unit. The acidic (pointed) end should be towards the positive electrode (RED).
· Follow the manual of the equipment and pay attention to page 6 of the **preparation manual**.

Separation of the second dimension

· Use precast gels, or prepare a 12.5% or gradient gel according to protein properties and sample complexity. If silver staining is required, make sure all solutions are filtered through 0.45μm membrane.
· Reserve one lane for the molecular weight markers.
· Incubate the strips in 1x sample Laemmli sample buffer for 15min. Use DTT as reducing agent.
· Place the strip on top of the stacking gel. Press down to make sure the lower edge of the strip contacts the stacking gel throughout, avoiding air bubbles trapped in between.
· Add molecular weight makers (for silver staining, 1ul of a 1/10 dilution of Benchmark)
· Run the gel according to manual until dye reaches the bottom.