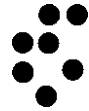


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TWO-DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS (2-DE)

We perform 2-DE on analytical and semi-preparative scale. Depending on protein sample complexity, we need 10-80 µg of a sample for analytical 2-DE and 100-500 µg for semi-preparative analysis.

First dimension - Isoelectric focusing (IEF)

IEF is performed on the **Ettan IPGphor II system** (GE Healthcare, Amersham Biosciences, USA). Proteins are separated on Immobiline DryStrip immobilized pH gradient (IPG) gel strips (GE Healthcare, Amersham Biosciences, USA), 3 mm wide, from 7 cm to 24 cm long, covering different pH ranges. The focused IPG strips are reduced and alkylated before running the second dimension.

Second dimension – Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE is performed on pre-casted or in-house casted PA gels, on analytical or semi-preparative scale, using the **Hoefer SE260 mini vertical electrophoresis unit** (Hoefer, USA) or the **Ettan DALT six unit** (GE Healthcare, Amersham Biosciences, USA). Protein spots are visualized by different staining procedures compatible with mass spectrometry analysis, such as Coomassie Brilliant Blue and silver staining or imidazole-SDS-Zn²⁺ reverse staining.

2-DE gel analysis

The stained gel is scanned by **Image Scanner using LabScan 5 software** (GE Healthcare, Amersham Biosciences, USA) and the scan is analysed by **Image Master 2D Platinum 6.0 software** (GE Healthcare, Amersham Biosciences, USA). Spots of interest could be excised from the gel manually or automatically using **Ettan Spot Picker** (GE Healthcare, Amersham Biosciences, USA).

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