SDS-PAGE

1. Purpose

To perform electrophoretic separation of complex protein mixtures under denaturing conditions to assist identification of proteins on the basis of molecular weight.

2. Procedure

2.1 Sample preparation

Thaw frozen protein extracts on ice. Prepare 2x reducing sample buffer (see Materials). Add at least an equal volume of 2x reducing sample buffer to protein extract to adjust protein concentration of each sample to 1 mg/mL. Boil samples containing 2x reducing sample buffer at 90^{o}C for 5 min then cool to room temperature. Centrifuge at 13000 rpm for 10 sec to collect condensation. Samples are then stored at -80 ^{o}C or analysed immediately by SDS-PAGE.

2.2 Running pre-cast polyacrylamide gels

Select percentage gel required for protein of interest and use appropriate buffer system throughout;

i) Use NuPAGE 4-12% Bis-Tris gels (Materials) to perform separation of all proteins with a molecular weight less than ~130kDa. (Buffer system A - Materials)
ii) Use 6% Tris Glycine gels (Materials) to perform separation of all proteins with a molecular weight greater than ~130kDa. (Buffer system B- Materials)

Remove gel from plastic pouch, take out comb and remove adhesive strip from foot of gel. Slide gels into Invitrogen Xcell SureLock Mini-cell (Materials) with wells facing into central chamber. Close the seal and fill central chamber with the appropriate running buffer (Materials). Add running buffer to outside chamber until buffer is higher than the foot of the gel. Ensure that seal is not leaking before loading samples.

The wells of a 10 well x 1.5mm gel can accommodate up to 60 uL of sample if loaded carefully with gel loading pipette tips (Materials). Flush out any residual storage buffer from the wells with running buffer before loading samples. Load 10 uL of Kaleidoscope pre-stained standards (Materials) in lane 1. Load 30 uL of each 1mg/mL protein sample (i.e. 30 ug).

Standard gel layout to minimise the impact of blotting artifacts on image analysis;

- Lane 1- 10 uL Kaleidoscope pre-stained standards
- Lane 2- wildtype 1
- Lane 3- mutant 1
- Lane 4- wildtype 2
- Lane 5- mutant 2
- Lane 6- wildtype 3
- Lane 7- mutant 3

- Lane 8- wildtype 4
- Lane 9- mutant 4
- Lane 10- 30uL 2x reducing sample buffer

Connect X-cell SureLock Mini-cell tank to power supply and run at 100V for 10 min, then 140V for 1 h 20 min, or until the dye front reaches the foot of the gel.

3. Materials

3.1 Chemical reagents

- 2x reducing sample buffer
    - To make 28.5 mL 2x sample buffer without reducing agent (store @ RT):
        - 1M Tris HCl pH 6.8 3 mL
        - 10% SDS 6 mL
        - 50% glycerol 9 mL
        - Bromophenol blue (BDH 200152E) solid to make it dark looking
        - Mill-Q H_20 10.5 mL
    - To make 1 mL 2x reducing sample buffer (store @ -20^oC)
        - 2x sample buffer without reducing agent 950 µL
        - 2-mercaptoethanol (Bio-Rad 161-0710) 50 µL

- Precision Plus Kaleidoscope standards (Bio-Rad 161-0375)

- Buffer system A – NuPAGE Bis-Tris
    - NuPAGE 4-12% gradient Bis-Tris gels 10 wells 1.5 mM (Invitrogen NP0335BOX).
    - To make 1 L 1x NuPAGE MOPS running buffer
        - 20x NuPAGE MOPS (Invitrogen NP0001) 50 mL
        - MilliQ-H_20 950 mL

- Buffer system B – Tris-Glycine
    - Tris-Glycine 6% gels 10 wells 1.5mM (Invitrogen EC6068BOX)
    - To make 1 L 10x Tris Glycine SDS running buffer
        - Tris (Amresco 0826) 30 g
        - Glycine (Sigma G8898) 144 g
        - 10% SDS 100 mL
        - Milli-Q H_20 to 1 L
    - To make 1L 1x Tris Glycine SDS running buffer
        - 10x Tris Glycine SDS running buffer 100 mL
        - MilliQ-H_20 900 mL

3.2 Equipment

- Heat block
- Benchtop centrifuge (eppendorf centrifuge 5415D)
- Gel loading pipette tips 1-200 uL (Fisherbrand FB56209)
- Xcell SureLock Mini-cell (Invitrogen EI0001)
- Power Pac (Bio-Rad)

- Zoom power supply adapters (Invitrogen ZA10001)

4. Quality Control

Commercially available pre-cast gels are used to help reduce the frequency of defective gels and improve reproducibility. Gel defects can be observed visually by studying the progress of the dye-front and the pre-stained protein standard ladder. If a significant gel defect is observed, the gel may be discarded.

5. Example Data

6. Supporting Information

7. Document History

This document was created by Rachel T. Uren on 22 February 2008.