Procedure 4: Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE) Analysis of Proteins

Reagents:
Acrylamide
N,N' Methylene-bis-acrylamide
Sodium dodecyl sulphate (SDS)
Ammonium persulphate
Tetramethylethylenediamine (TEMED)
Tris(hydroxymethyl)aminomethane (Tris)
Hydrochloric acid (HCI)
Glycerol
2-Mercapthoethanol
Bromophenol Blue
Deionised water

Materials & Equipment:

Latex gloves
Weighing balance
Graduated cylinder (200ml)
Light-proof container (200ml)
Fume cupboard
Electrophoresis Apparatus
Power Pack
Pipettes
Automatic Pipette Aid
Hamilton syringe
Eppendorf Tubes
Piercing Implement

Materials & Equipment (continued)

Boiling water bath
Magnetic stirring bar and stirrer
Universal containers (25ml)
Graduated cylinders
Beakers

Reagent Preparation:

A. 1.5M Tris-HCI Buffer, pH 8.8 (100 ml)

A.1 Dissolve 18.15g of Tris in 75ml deionised water using a magnetic stirrer. Adjust pH to 8.8 using 5M HCI and make up the total volume to 100ml using deionised water.

B. 0.5M Tris-HCI Buffer, pH 6.8 (100ml)

B.1 Dissolve 6.05g of Tris in 75ml deionised water using a magnetic stirrer. Adjust to pH 6.8 using 5M HCI and make up the total volume to 100ml using deionised water.

C. 10% (w/v) Sodium Dodecyl Sulphate (10ml)

C.1 Dissolve 1g of SDS in 8ml deionised water and make up to 10ml with deionised water. Store at room temperature for up to 6 months. If precipitation occurs during low temperature storage, incubate the mixture at 37°C until SDS is resolubilised.

D. 10% (w/v) Ammonium Persulphate (1ml)

D.1 Dissolve 0.1g Ammonium Persulphate in 1ml deionised water in an eppendorf tube.

E. 30% (w/v) Acrylamide/Bis (200ml)

E.1 Weigh out 58.4g acrylamide and 1.6g N,N'-methylene-bis-acrylamide. ***Caution! Acrylamide is extremely toxic.*** Wear gloves and face mask.

E.2 Add the two above reagents into a beaker and add 200ml deionised water. Dissolve using magnetic stirring bar and stirrer.

E.3 Store the acrylamide/bis solution in a light proof container at 2 - 8°C.

F. 0.5% (w/v) Bromophenol Blue Solution (20ml)

F.1 Dissolve 0.1g bromophenol blue in 20ml deionised water.

G. Solubilisation Buffer 5X

G.1 Weigh out 10g (8ml) of glycerol into universal.

G.2 Add 4ml of deionised water, 1.6ml of 10%(w/v) SDS and 1ml of 0.5M Tris-HCI buffer, pH 6.8.

G.3 Add 0.4ml 2-mercapthothanol to the above solution (inside a fume cupboard) and finally add 0.2ml of bromophenol blue solution.

H. Electrode (Running) Buffer 5X (5 litre)

H.1 Add 75g Tris, 360g glycine and 25g SDS to 4000ml deionised water in a 5 litre graduated cylinder and dissolve using a magnetic stirrer. Once dissolved make up to 5 litres with deionised water. This is a 5X buffer concentrate.

H.2 Before electrophoresis, the running buffer is prepared by diluting 200ml of the above concentrate with 800ml deionised water.

I. Electrode (Running) Buffer 5X (1 litre)

Note: 15g Tris, 72g glycine and 5g SDS to 1000ml deionised water in a 1 litre graduated cylinder and dissolve using a magnetic stirrer. Once dissolved make up to 1 litres with deionised water. This is a 5X buffer concentrate. Store at room temperature. Stable for up to 6 months.

Before electrophoresis, the running buffer is prepared by diluting 200ml of the above concentrate with 800ml deionised water.

Procedure:

Gel Casting

	Stacking Gel	Separating Gel Concentration	
	4%	7.5%	12%
Reagent	**Volume**	**Volume**	
(30% (w/v)) Acrylamide bis (ml)	**1.3**	**2.5**	**4.0**
Tris-HCI, pH 8.8 (ml)	**-**	**2.5**	**2.5**
Tris-HCI, pH 6.8 (ml)	**2.5**	**-**	**-**
Deionised water (ml)	**6.1**	**4.85**	**3.5**
(10% w/v) SDS (μl)	**100**	**100**	**100**
Ammonium persulphate (10% wv) (μl)	**200**	**200**	**200**
TEMED (μl)	**20**	**20**	**20**

The SDS polyacrylamide gels are cast as follows:

Note: Gloves must be worn at all stages to avoid skin contact with the neurotoxic acrylamide.

(The manufacturers instructions for the mini-protean 11(Biorad) gel casting and electrophoresis apparatus, and the powerpack unit 9 model 200/2, Biorad).

I.1. The separating gel of the desired concentration is prepared in a 25ml Universal container following the above formulation. The TEMED and ammonium persulphate should be added immediately prior to pouring the reagents into the casting chamber. Ensure reagents are mixed before pouring. Allow about 1 - 1.5cm above the top level of the poured gel for the addition of the stacking gel. Layer the poured running gel with deionised water until polymerised and remove immediately before pouring of the stacking gel.

I.2. After polymerization, prepare the stacking gel as formulated above, again add the TEMED and ammonium persulphate immediately before gel pouring. Pour into the space above the running gel and immediately insert the well template.

Electrophoresis

J.1. Remove the well template from the stacking gel and fit the casting unit to the electrode unit.

J.2. Place the unit in the electrophoresis tank and fill the central reservoir with 300ml Electrode Running Buffer **(H)** allowing it to overflow into the tank base.

J.3. To every four volumes of sample add 1 volume of 5X Solubilisation Buffer **(G)** in an eppendorf tube. Pierce the tube cap and boil the mixture for 5 minutes.

J.4. Using a Hamilton syringe, add up to 30µl of the boiled sample per well as required.

J.5. Connect the electrophoresis apparatus to the power pack and electrophorese at 200V until the tracking dye reaches the base of the gel.

J.6. Upon completion of electrophoresis, remove gels from between glass plates and stain / blot as required.

J.7. Clean all electrophoresis apparatus and store at room temperature.