2D-PAGE

(1) First Dimensional Isoelectric Focussing (IEF)

1.1 Materials.

a. 30% acrylamide stock solution

Acrylamide (28.38%)	28.38 g
BIS (1.62%)	1.62 g

Make the volume to 100 ml with water

Acrylamide is a potent neurotoxin and should never be mouth pipeted. Acrylamide solution is light sensitive and should be stored in the dark at 4 ^{o}C.

b. Anode electrode solution

0.02N H_3PO_4

(1M stock: 6.364 ml phosphoric acid to 100 ml water)

c. Cathode electrode solution

0.02 N NaOH

(1M stock: 4 g Sodium Hydroxide to 100ml water)

d. SDS Sample buffer (pH 6.8)

Tris-HCl (0.06 M)	3.785 g
SDS (2%)	10 g
β-mercaptoethanol (5%)	25 ml
Glycerol (10%)	50 ml

Make the volume up to 500 ml with water (MQ)

1.2 Method

a. Mark 2cm from the top of clean, dry 13 cm long and 3 mm diameter, 1d gel tubes to indicate the desired height of the gel. Place a rubber band around them so that they form a tight bundle. Hold the bundle vertically on a flat surface. Seal the bottom with two layers of parafilm

b. Prepare the solution

Urea	4.8 g
30% acrylamide stock	1.16 ml
NP-40	2 ml
Water	2.84 ml
Ampholines (pH5-7)	0.25 ml
Ampholines (pH 3.5 –10)	0.25 ml
10% APS	15 µl
TEMED	10 µl

Note. Dissolve the urea by swirling in water bath whose temperature should not higher than 60^{o}C

c. Using a long narrow gauge hypodermic needle, fill the gel tubes with the solution

of step 3 to the mark.

d. Overlay the gel mixur with 20 µl of water and allow it to polymerize it for 1 h.

e. Remove the para-film from the bottom of the tubes and the water from the top of the gel.

f. Seat the tubes in the holes of the upper buffer reservoir of the tube cell. Plug any unused hole with rubber stopper

g. The lower chamber is filled with 0.02 N Phosphoric acid . The samples are loaded in a volume 5 – 100 µl and overlaid with 20 µl of 1/2 lysis buffer. The upper chamber is filled with 0.02 N Sodium Hydroxide.

h. Run the current as follows

200 V	for	30 min
400 V	for	16-18 h
600 V	for	1 h

i. Extrude the gel from the tubes using the water pressure from a syringe. Wash each gel in 5 ml SDS sample buffer for 15 minutes. Change the buffer and wash it again for 15 minutes. At this point, the gel can be stored at –20^{o}C or used immediately.

(2) First Dimensional Immobilized pH Gradient (IPG)

a. Seat the tubes in the holes of the upper buffer reservoir of the tube cell. Plug any unused hole with rubber stopper

b. The lower chamber is filled with 0.02 N Sodium Hydroxide.

c. The water is removed from the top of the gel. The samples are loaded in a volume 5 to 100 µl with a syringe and overlaid with 20 µl of 1/2 lysis buffer. The upper chamber is filled with 0.02 N Phosphoric acid. Connect positive terminal with lower chamber (acidic) and negative terminal to upper chamber (basic).

Run the current as follow

400 V for 1 h

1,000 V for 16 h

2,000 V for 1 h

d. Remove the tubes and force the gels out onto a parafilm. Wash each gel in 5ml SDS sample buffer for 15 minutes. Change the buffer and wash it again for 15 minutes. At this point the gel can be stored at –20^{o}C for several week or used immediately

(3) Second Dimension with IEF and IPG

3.1. Materials

a. Separating gel buffer (pH 8.8)

Tris HCl (1M)	12.11 g
SDS (0.27 %)	0.27 g

Make the volume to 100 ml with water (MQ)

b. Stacking gel buffer (pH. 6.8)

Tris HCl (0.25 M)	3.03 g
SDS (0.2%)	0.2 g

Make the volume to 100 ml with water (MQ)

c. Acrylamide for separating gel

Acrylamide	30 g
BIS	0.135 g

Make the volume to 100 ml with water (MQ)

d. Acrylamide for stacking gel

Acrylamide	29.2g
BIS	0.8g

Make the volume to 100 ml with water (MQ)

e. 1% agarose

1 g agarose /100 ml water

f. Running buffer

Tris-HCl	9.0 g
Glycine	43.2 g
SDS	3.0 g

Make the volume to 3 L with water (MQ).

g. Bromophenol blue solution

BPB 0.01 g /100 ml of 10 % glycerol

3.2. Method

a. Assemble detergent cleaned gel plates. The separating gel is cast first, followed by the upper stacking gel. For a gel with dimension 0.75 mm x 14cm x 14cm, about 20 ml of separating gel mixture and about 5ml of stacking gel mix is needed.

b. Prepare 15% polyacrylamide gel as shown below

	For one gel
Acryalamide for separating gel	8.5 ml
Separating gel buffer (pH.8.8)	6.3 ml
Water	2.0 ml
10% APS	120 µl
TEMED	20 µl

Swirl to mix and pour the gel immediately. Gently overlay with 1cm of water. Allow the gel to polymerize for 45 to 60 min

c. Preparation of Stacking gel solution

Acrylamide for stacking gel	1.0 ml
Stacking gel buffer	3.0 ml
Water	2.0 ml
APS	30 µl

TEMED	20 µl

For one gel

Remove the overlaid water and pour the stacking gel solution immediately. Leave it to polymerize for 10-15 minutes

d. The first dimension is applied directly on the top of the stacking gel. The first dimension is overlaid by 1% agarose.
e. The slab gel is assembled for running. A few drops of tracking dye (bromophenol blue) is added
f. Run the sample at 35 mA (constant current) until the tracking dye reaches the bottom of the separating gel.
g. Separate the gel plates and either stain the gel or process for western blot. The gel is stained either with silver staining or Coomassie Brilliant Blue