**SDS-Poly acrylamide gel electrophoresis (SDS-PAGE)**

In this system, two sequential gels are actually used; the top gel, called the stacking gel, is slightly acidic (pH 6.8) and has a low acrylamide concentration to make a porous gel. Under these conditions proteins separate poorly but form thin, sharply, defined bands.

The lower gel, called the separating (resolving gel) is more basic (pH 8.8), and has a higher polyacrylamide concentration which causes the gel to have narrower channels or pores.

As a protein, concentrated into sharp bands by stacking gel, travels through the separating gel, the narrower pores have a sieving effect, allowing smaller proteins to travel more easily, and rapidly than larger proteins.

**Gel preparation**

* Separation gel contents

|  |  |
| --- | --- |
| components | Amount  |
| 1.5 M Tris-HCL PH 8.8  | 2 ml |
| H2O | 2.8 ml |
| 10% SDS  | 80µl  |
| 10% Ammonium persulphate (fresh) | 100 µl |
| TEMED | 20 µl |
| Acrylamide stock | 3.2 ml |

* Stacking gel contents

|  |  |
| --- | --- |
| components | Amount  |
| 1.5 M Tris-HCL PH 6.8  | 1 ml |
| H2O | 3 ml |
| 10% SDS  | 80 µl |
| 10% Ammonium persulphate (fresh) | 100 µl |
| TEMED | 20 µl |
| Acrylamide stock | 1 ml |

* SDS-PAGE Running buffer pH 8.4 (5 X)

|  |  |
| --- | --- |
| components | Amount  |
| Tris | 15 g |
| Glycine  | 72 g |
| SDS | 5 g |
| Made up to 1L with distilled water |

* SDS- PAGE Disruption buffer

|  |  |
| --- | --- |
| components | Amount |
| 20% (w/v) SDS  | 1 ml |
| 1M Tris HCL pH  | 0.5 ml |
| Glycerol  | 1 ml |
| B- mercaptoethanol  | 0.5 ml |
| Bromophenol blue | 0.01 g |
| Made up to 10 ml with distilled water |

**Sample Preparation**

For best results, all samples should be in identical, low ionic strength buffers.

1. Mix 40 μl of each sample with 10 μl of disruption buffer.
2. Heat in a boiling water bath in for 2 min. in most cases, brief boiling 3 min improves denaturation, but it may also cause the protein to precipitate.

**Electrophoresis**

1. Remove the comb and clamp the gel to the electrophoretic apparatus.
2. Fill thr top electrolyte compartment with running buffer.
3. Check for leaks from thr top into bottom compartment, if there are no leaks, fill the bottom compartment.
4. With a plastic Pasteur pipette, thoroughly rinse each well in the stacking gel with running buffer.
5. Apply the sample by using a micropipette to carefully add up to 25 μl of protein to bottom of a well.
6. Replace the cover of the electrophoretic cell, with the (+) symbol on the cover connected to the (+) on the cell, so that the anode (+) is the bottom electrode.
7. Apply 15 mA/gel until the oroteins are well into the stacking gel, then 35mA/gel until the tracking dye reaches the bottom of the gel ( about 45 min).
8. Always turn down the power and unplug the wires from the power supply before removing the cover.

**Protein staining procedure**

* SDS-PAGE STAIN

|  |  |
| --- | --- |
| components | Amount  |
| Glacial acetic acid | 70 ml |
| Ethanol  | 400 ml |
| Coomassie blue | 0.25 % |
| Made up to 1L with distilled water |

* SDS-PAGE destain

|  |  |
| --- | --- |
| components | Amount  |
| Glacial acetic acid | 70 ml |
| Ethanol  | 400 ml |
| Made up to 1L with distilled water |

1. Drain excess buffer from the gel and rinse in wash solution to remove SDS and fix the proteins.
2. Rock the gel in the wash solution for 15 min , then remove and discard the solution.
3. Add enough staining solution to cover the gel . stain for an hour.
4. Remove the staining solution and replace with 100 ml wash solution.
5. Swirl the wash over the gel by racking the covered container for several minutes/ hours or until excess stain is removed and unstained areas are completely clear.
6. Photograph, interpret visually, or quantitate using appropriate densometric equipment