Protocol: SDS-PAGE Gel Electrophoresis

Recipe for (one) 15% Gel

Component	Resolving	Stacking
Lower Gel Buffer*	1.25 ml	-
Upper Gel Buffer*	-	1.25 ml
Deionised Water	1.825 ml	3.3 ml
Acrylamide Solution**	1.875 ml	400 µl
APS***	25 µl	50 µl
TEMED*	2.5 µl	5 µl

* These components are in the cupboard marked Gel Electrophoresis
** This component is in the fridge
*** This component is in the freezer (-20ºC)

Preparing the Resolving Gel

1. Once you have assembled the glass plates in the gel casting machinery you are ready to cast your gel.

2. Make sure that you have the necessary component for the resolving gel in front of you.

3. In a 15 ml facon tube, mix the first four components of the resolving gel.
   - Remember that this is enough for only one gel, double the amount for two (resolving gel only)!

4. Allow the APS to thaw and add the appropriate amount the above mixture.

5. Finally add the TEMED.
   - TEMED initiates the polymerisation of the gel- so once you add it, you <u>must</u> work quickly!

6. Once you have added the TEMED, use the P1000 to mix the solution thoroughly.

7. Still using the P1000, apply the resolving gel solution between the casting plates.
   -Tip: apply the solution at the corner of the two plates – this will avoid air bubbles!

8. Once the gel solution has been applied (to the green mark), add deionised water on top.
   -Air prevents the polymerisation of the gel.

9. Leave gel to set for about 30 minutes.

- There will be some gel solution in the falcon tube. You can use this to check that your gel has set.

Preparing the Stacking Gel

1. Remove the water above the cast gel (above) and continue to prepare the stacking gel.

2. Mix ingredients for the stacking gel and proceed as above, using the P1000 to apply the solution.

3. Once you have applied the stacking gel, immediately insert the green well comb.

4. Again leave to polymerise for approximately 30 minutes.

Preparing Your Samples

1. If your sample is in liquid form, aliquot a small amount (20-50µl) into a fresh eppendorf and mix in a 1:1 (v/v) ratio with Laemilli Buffer (in the freezer -20^{o}C). Continue to step 3.

2. If your sample is in solid form (bacterial pellet or lyophilised protein) then add 50µl of an appropriate buffer (Tris) and re-suspend or re-dissolve the solidified samples. Vortex for 15 seconds at maximum setting. Centrifuge the samples (Minispin) at 13,000rpm for 60 seconds. Use the supernatant and follow step 1 above.

Loading the Gel

1. Carefully remove the comb from the gel and wash wells with deionised water. Remove excess water using paper towels.

2. Remove plates from casting assembly and transfer to the electrophoresis assembly and tank.

3. Add about a litre of running buffer into the tank, making sure that the wells are fully covered. You are now ready to load your samples onto the gel.

4. Attach the yellow well marker to the electrophoresis assembly. Take the P20 and aliquot 10 µl of the Protein Marker (PM, in the fridge, Invitrogen) and load into the first well. This must be done slowly to avoid contaminating other wells. Continue loading your samples into the wells, noting the well number and sample loaded as you go.

Running the Gel

1. Take the tank with gel into the cold room (2nd floor).

2. Put the lid onto the tank (red with red).

3. Set the volts to be 150V and press run.

4. It should take about 1 hour.

Making the Bands Visible

1. Remove the gel from the apparatus and wash with water.

2. Carefully remove the gel from the plates and soak in fixing solution* for 20 minutes (agitate on the belly dancer).

3. Exchange the fixing solution for the Coomassie stain* and soak for 1 hour or overnight (agitate on the belly dancer).

4. Destain with destaining solution* until gel is clear or overnight (agitate on the belly dancer).

*These solutions are reusable and are kept in the Gel Electrophoresis cupboard.