BIOTECHNOLOGY Labclass No.5

POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

Unlike the electrophoresis we have done so far, in the case of PAGE, the samples will "run from top to bottom", ie vertically.

1) Cleaning the apparatus with denatured alcohol. Pair of glasses will be inserted into the basic frame, between the glassess the gel will be poured.

2) A pair of glasses, one of which is thin and the other thick, is inserted in the green box. (The ridges of thick glass create a gap for the gel).

3) Attaching the green frame with the glass to the apparatus. Attach the frame to the basic apparatus so that it rests on a silicone or rubber pad and press it firmly against the pad. (This will prevent the gel from leaking).

For PAGE it is necessary to use two gels, a resolving gel at a concentration of 12% and a stacking gel at a concentration of 4%. The distribution and concentration gel includes a premix containing a mixture of acrylamide and bisacrylamide, as well as Tris buffer supplemented with HCl to the optimal pH and SDS.

APS (ammonium persulphate) and TEMED (tetramethylethylenediamide) must be added to the premix.

The concentration gel is important for the concentration of the sample (it forms the upper layer) and the lower layer forms a distribution gel, thanks to which the proteins are divided according to molecular weight.

Mix each gel with other components in 15 mL tubes:

4) For one dose of distribution gel (12%) mix: Mix gently so that no bubbles are formed.

7mL premix

80µL APS

7µL TEMED

1) Pipette 4.5 - 5 ml of distribution gel between the glasses, leave the rest of the gel in a test tube so that we can monitor its solidification.

2) Overlay the gel with about 350 µl of isopropanol to equalize the gel level.

3) Check that the rest of the gel in the test tube has solidified, if so we can continue to apply the concentration gel

4) For one dose of concentration gel (4%) mix:

4mL premix

50µL APS

4µL TEMED

5) Pipette the concentration gel just below the upper edge of the glasses onto the solidified distribution gel, from which we aspirated isopropanol with a paper towel, leaving the rest of the gel in a test tube so that we can monitor the solidification. Before the concentration gel hardens, place a plastic comb between the glasses.

6) Wait for the concentration gel to solidify.

7) Pull the glass out of the frame and carefully remove the comb. Insert slides with solidified gel into the electrophoresis frame. Snap carefully so that the frames enclose the slides tightly so that there are no gaps through which the buffer can flow.

8) Place the frames with slides in an electrophoretic bath and pour the electrophoretic buffer. Electrophoretic buffer is obtained by diluting 10x concentrated running buffer.

9) Sample preparation: mix 40 µL of bacterial lysate with a loading dye (10 µL). The dye contains: SDS; mercaptoethanol; glycerol and bromophenol blue. SDS acts as a denaturing reagent, mercaptoethanol as a reducing agent to break sulfide bridges, bromophenol blue for staining and glycerol to lower the sample to the bottom of the well.

Physical and chemical denaturation of the proteins takes place in a thermoblock for 5 minutes at 70 ° C, which ensures denaturation of the protein.

10) After pouring the slides in the electrophoretic bath with electrophoretic buffer so that the buffer extends beyond the edges of the slides, apply the samples with a pipette with special tips in an amount of 30 µl to each well.

One gel can hold 1 ladder and 9 samples. Ladder (4 µl) or protein standard is applied first from the left. Carefully insert the narrow tip between the glasses and apply the sample.

11) Put on the lid of the electrophoretic bath with electrodes, connect it to the power supply and turn it on. The electrophoresis parameters are set to 180 V / 400 mA / 30 minutes.

12) At the end of the electrophoresis, remove the glasses and break them with a spatula so that we can remove the gel and stain it. Using a spatula, transfer the gel to a tray of staining solution. The gel is then stained with a mixture of Coomasie blue, acetic acid and methanol and then decolorized with a mixture of acetic acid and methanol.