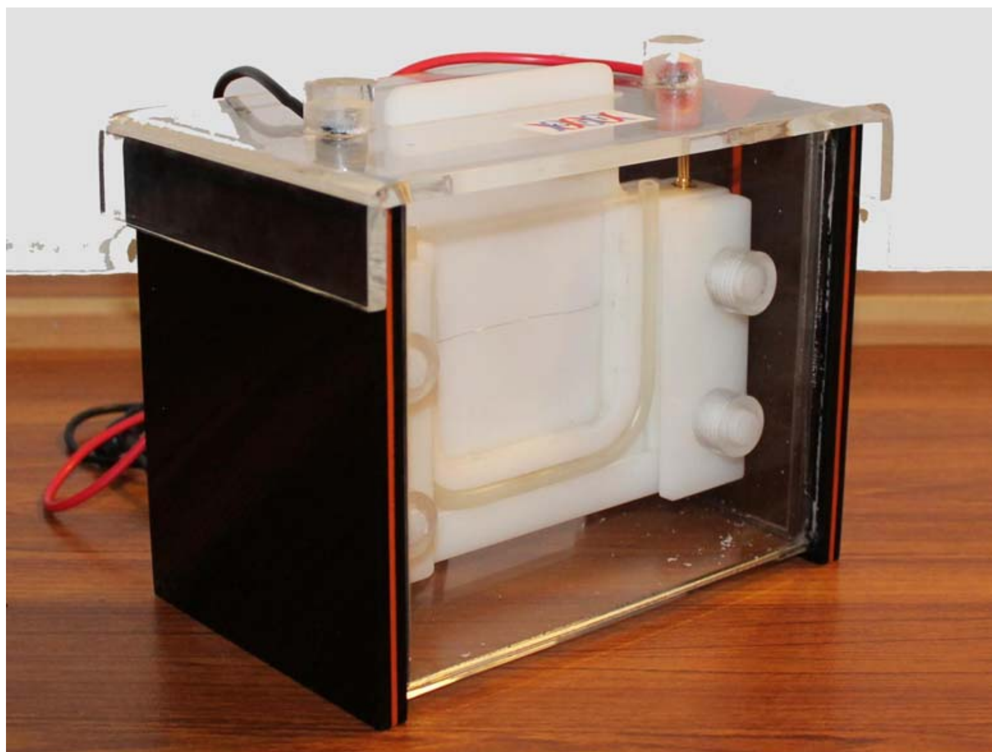
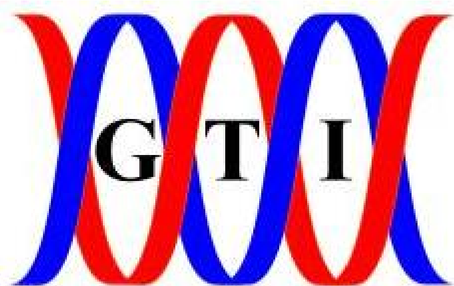
GTI-Mini/Midi PAGE Apparatus

Instruction Manual





Genetic Technology Instrumentation

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Electrophoresis is used for the separation of charged molecules like DNA, RNA, or proteins by an electric current applied to a gel matrix. In PCR applications it is usually done for separation and visualization of DNA after PCR or sequencing. The electrophoresis gel is a porous matrix that allows movement of DNA through its pores. DNA fragments with large differences in size are typically separated on agarose gels whereas small differences in DNA fragment sizes are resolved on polyacrylamide gels. The nucleic acids have a negative charge due to the sugar phosphate backbone of the molecule. This allows movement from the negative to the positive electrode. The double-stranded DNA fragments naturally behave as long rods, so their migration through the gel corresponds to their size.

Acrylamide is a white crystalline powder that forms polymers after dissolving in water. The acrylamide polymers can be cross-linked by addition of bisacrylamide. As a result of cross-linking the pore size of the gels also decreases. This makes acrylamide gels most suitable for separation and resolution of DNA molecules that may differ in size by only one base pair. The acrylamide gels can withstand high temperatures and hence are suitable for applications requiring high voltage. Acrylamide gels are typically run in a vertical position using discontinuous buffer compartments.

Acrylamide in solution becomes viscous but does not form a gel. The cross-linked acrylamide polymers can be solidified to form a gel by adding ammonium persulphate (APS) and N,N,N',N'-tetramethylethylenediamine (TEMED) in equimolar ratio. The concentration of APS and TMED determine the rate of gel formation and its turbidity and elasticity.

Acrylamide should be stored in a cool dark and dry place to reduce autopolymerisation and hydrolysis. Acrylamide is neurotoxic that is absorbed through skin. Its effect is cumulative and toxicity may develop over prolonged exposure. Gloves must be worn when using acrylamide.

Assembling Gel Plates

1. Clean the glass plates, spacers and combs with light detergent.
2. Carefully wipe the water with soft cloth and leave the plates to dry in upright position.
3. Before assembly wipe the two plates with soft cloth rinsed in methanol.
4. Place the large glass plate on clean level bench and position the two side spacers aligned with its edges. Overlay the small (notched) plate.
5. Carefully assemble the glass plates in the gel running unit and gently tighten the screws. Over tightening of the screws or mal-aligned plates may result in breakage of the glass plates.
6. Place the gel running unit in the casting stand placed on horizontal flat surface.

Pouring the Gel:

1. Seal the bottom end of the assembled glass plates by pouring the sealing mix (1.5 ml 6% acrylamide solution, 50 µl 10% ammonium persulphate (APS) and 10 µl TEMED). The sealing mix should be poured quickly as it can polymerize in seconds.
2. Allow 2-3 minutes for the sealing mix to polymerize.
3. For the GTI-Mini PAGE gels take 5 ml 6% acrylamide solution in a small beaker and add 50 µl 10% APS and 10 µl TEMED.
4. For the GTI-Midi PAGE gels take 12 ml 6% acrylamide solution in a small beaker and add 110 µl 10% APS and 25 µl TEMED.
5. Gently pour the acrylamide solution between the glass plates with a 10 ml syringe. Butterfly or ordinary needle may be used to allow smooth filling. Care should be taken to avoid any bubbles. Any bubbles formed may be allowed to escape by gently tapping the plates.
6. Gently place the comb at the top of the filled gel plates.
7. Leave the gel to polymerize for at least 15-20 minutes.
8. Carefully remove the comb when the gel has polymerized.
9. Wash the wells by gently flushing these with buffer. A syringe and butterfly needle may be used for flushing the wells.
10. Load 3-5 µl of the amplified DNA mixed with loading dye.
11. Place the gel running unit in the buffer tank.
12. Fill the two buffer compartments with adequate amount of gel running buffer.
13. Placing the lid in the correct position automatically aligns the cathodal and the anodal connections.
14. The Mini gel is run for 30-40 minutes at 150-200 volts or as desired.
15. The Midi gel is run for 1-3 hours at 150-200 volts depending on the amplified product size.
16. Correct electrical connections results in rising of a stream of bubbles in the upper buffer compartment.
17. Disconnect the power supply at the end of the electrophoresis run.
18. Carefully open the lid and remove the gel running unit out of the buffer tank.
19. Remove the gel plates from the gel running unit by opening the screws.

20. Very carefully split open the two glass plates and mark the first well on the gel by cutting the corner of the gel.
21. Gently transfer the gel to a staining tray taking care not to damage the fragile gel.

Silver staining of the gel

1. Put the gel in 0.1% silver nitrate solution for 15-20 minutes. The gel may be kept in stain while it is still on the glass plate.
2. Discard the stain and wash the gel in plenty of tap water.
3. The stain may be reused if kept in dark brown bottles. However it looses potency after exposure to bright light.
4. Prepare fresh developing solution by adding 75 µl formaldehyde to approximately 100 ml 1.5% NaOH. Stock NaOH is stable at room temperature but once formaldehyde is added it should be used within a few hours.
5. Submerge the gel completely in developing solution.
6. In approximately 5-10 minutes the bands of amplified DNA can be seen on the gel. The background of the gel also becomes light yellowish brown. The background colour could become very dark if the gel is kept for too long in the developer solution. The exact developing time can be learnt by trial and error.
7. Wash the gel in plenty of water when the DNA bands are clearly seen.
8. Cut a piece of filter paper slightly larger than the gel itself and lay it flat on the gel surface. Gently pick the filter paper along with the gel that sticks to its surface.
9. Place the gel and the filter paper on a gel dryer making sure that the gel faces towards the front.
10. Dry the gel under vacuum for 20-30 minutes at 80°C.
11. The dried gel can be pasted in a record book for long term storage after trimming its margins.
12. The gel may be photographed if a gel dryer is not available.