**Preparation of Polyacrylamide Gel for PAGE**

Column A

Support for plates

Column B

1. Remove the existing solution (1X TBE Buffer) from Column A into an empty glass bottle (Duran Bottle), with the help of tube attached with column.

Glass Plate B

Glass Plate A

1. Unscrew the support for the glass plates to take out the plates from the apparatus.
2. Gently take the plates out and take them to the sink. Remove the spacers from each side and from one corner, slowly detach the two plates.
3. Using a spacer/ruler, remove the existing gel from the plate, and wash the two plates with tap water followed by rinsing with distilled water.
4. Keep the washed plates for drying, or wipe the water using tissue paper.
5. On two slabs, place the glass plate A (the plate with groove) and using a pipette, pour 20µL of silane (kept in fridge) on the area where the wells will be engraved later, as a horizontal line.
6. Take a Kimwipe and add some 90% ethanol to it. Use this to wipe the area on the plate where silane was poured. Wipe for some time till it dries.
7. Again, pour 90% ethanol over that area, and wipe again, as we do not want too much concentration of silane in that area.
8. Take this plate A and keep it on side.
9. Now, place the glass plate B on the slab. Add three spacers, two on sides and one at the bottom edge. Over the spacers and plate B, place the plate A, such that the side with silane is faced inside (towards plate B).
10. Use three clips, two at sides and one at bottom, to hold the plates together. The spacer at the bottom edge should not be completely over the edge, try to place it partially on edge and partially outside.
11. Place the 100 wells comb on the upper side, partially inserted between the two plates.
12. Then, using a measuring cylinder (100mL capacity), take 80mL of 6% gel (kept in brown bottle in the fridge) in a plastic beaker.
13. To this, add 400µL APS (kept in fridge in separate Eppendorf tubes) followed by addition of 56µL TEMED solution (kept on the rack above microwave). Stir the contents of the beaker properly.
14. Take a glass pipette and attach it to an instrument called pipetter. Switch it on and press the up button to take the liquid from beaker into pipette.
15. Place the pipette over the well region of the comb and press down button to release the liquid. Use a scissors to tap simultaneously on the plates such that no air bubbles are created.
16. Use a plastic thin spacer or ruler to remove any bubbles, by putting it in between the plates.
17. After adding the gel between the plates, use more clips to hold the plates together.
18. Completely insert the comb between the two plates and hold with clips.
19. Allow the gel to solidify for 40 minutes.
20. After solidification, remove all the clips and take the plates to the sink. Remove the bottom spacer carefully and rinse the plates with distilled water. Wash outside of the plates as it’ll remove any solution marks/stains on the plate.
21. Use any sharp instrument and try to cut the gel between the edge of the comb and plates, so as to remove the comb afterwards. Gently remove the comb and rinse the plates again.
22. Wipe the water from outside the plates and place it in the apparatus in standing position (put it over a special space inbuilt in the column B). To hold the plates, use the support and screw (not too tightly as it might break the plates).
23. Add 1X TBE solution in Column B till the point that it doesn’t immerse the glass plates.
24. Take 200µL EtBr (ethidium bromide, kept in the rack above microwave), and pour In column B equally from one end to another. Mix with the same pipette tip.
25. Cover the column B with Anode lid.
26. Now, add 1X TBE buffer in the column A, such that it immerses the wells of the glass plate completely, and doesn’t let the gel dry.
27. Using a multi-tip pipette, load samples (10µL) into the well from the top. While loading the samples, the tip should completely go inside the wells and when releasing the sample, keep taking out the tip from the wells, so that the space is covered by the sample.
28. Don’t release the pipette until all the tips are out from the wells, to avoid air bubbles. Release the pipette and wipe on tissue paper.
29. After loading all the samples, load 10µL of ladder (100 bp in this case) in one of the starting wells or the end wells.
30. Cover column A with the Cathode lid.
31. Switch on the electrophoresis apparatus, select ‘Manual’, set voltage to 300 V and time to 2.5-3 hrs, and press run.