

1. Assemble the gel mould:

WHAT YOU NEED
Glass plate to fit apparatus
Silane A174 in Ethanol Acetic Acid
Silicone rubber cord 2 mm
Well moulding plate: perspex
Clips/Clamps - 4 sizes

1. Treat the glass plate with Silane A174 and dry. (See item 2)
2. Feed the cord through a gap in the teeth of the mould and clip it at the other side of the teeth, leaving a 'tail' long enough to reach the other end of the mould.
3. Pass the cord behind the teeth and feed it through a second gap, leaving the appropriate number of teeth to form the required number of wells.
4. Lay the glass plate on the silicone rubber cord, with the treated surface against the cord.
5. Apply clips to apply pressure to the silicone rubber cord behind the teeth.
6. Pull on one end of the cord to straighten it and clip to apply pressure to cord.
7. Repeat with the other end of the cord to create a 'U' shaped seal between the mould and the glass plate.
8. Making sure that the pressure of the clips is applied directly over the cord, use as many clips as needed to form a seal around the cord.

2. Pre-treatment of glass plates:

WHAT YOU NEED
Glass plates to fit electrophoresis apparatus
Silane A174 working solution ¹
Lint free tissue

1. Make up the Silane A174 working solution¹.
(Silane A174 is 3-trimethoxysilyl-propyl-methacrylate)
2. Thoroughly wash the plate to be treated. Take care to scrape off any gel fragments attached to the plate from previous gels.
3. Dry the plate using a lint-free tissue or leave to air dry.
4. Pipette 2-4 ml (depending on plate size) of the Silane solution onto the plate and distribute equally over the plate with a lint-free tissue. Cover the plate to prevent dust contamination and leave to air dry on the bench for 1-1.5 hours
5. Polish the plate with a lint-free tissue, moistened with a small amount of double-distilled water or ethanol.
6. The plate is now ready to pour the gel.

Notes:

¹ Silane A174 Working Solution: Ethanol 8 mL, Glacial Acetic acid 200 µL, Silane A174 10 µL, distilled H₂O 1.8 mL

3. Prepare the gel mix:

WHAT YOU NEED
Acrylamide/bis monomers, 19:1, 40% w/v
Ammonium persulphate, 10% w/v
TEMED
Buffer Stock - 10X TBE

1. The acrylamide monomer stock solution is 40% w/v; the buffer stock is 10X. Calculate appropriate amounts for the requisite volume of gel². This mix can be stored at room temperature or in the fridge.
2. For each mL of gel mix, add ~10 µL 10% ammonium persulphate³ and 2 µL TEMED.
3. Pour the mix into the mould immediately.

Notes:

² Volume in mL = width (cm) x length (cm) x 0.2; allow some extra in case of leakage

³10% ammonium persulphate stock should be made fresh each month and stored in the fridge. The amount of ammonium persulphate needed depends on ambient temperature. The amount suggested here is for a temperature around 20 deg C. (Ammonium persulphate crystals are hygroscopic, so store the bottle in a desiccator).

4. Pour the gel:

1. Tilt the moulding assembly so that it rests on one corner.
2. Pour the gel mix slowly into the mould so that solution rises behind the row of teeth. Make sure that no bubbles or pockets of air are trapped behind or between the teeth.
3. Once the gap behind the teeth is filled, lower the bottom corner so that the assembly rests on its end.
4. Continue pouring gel mix until the desired height is reached⁴.
5. When the gel is set, remove the moulding piece from the glass plate.

Take care not to tear the sides of the wells - lift the mould without lateral movement.

5. Run the gel:

1. Lay the plate/gel in the horizontal apparatus.
2. Overlay with running buffer⁵ to about 2 mm over the gel.
3. Apply the samples in loading buffer (1-8 µL) as for agarose gel electrophoresis.
4. Apply potential for the appropriate run time.

Notes:

⁴In case there is leakage past the seal, lower the assembly, to near horizontal, once the mould is filled. Top up as necessary.

⁵Running buffer can be diluted with bottled drinking water.

6. Stain and photograph:

WHAT YOU NEED
EtBr in water or electrophoresis buffer (0.5 µg/mL OR 0.1 µg/mL) ⁶
2.62 mm silicone rubber tubing
Staining tray OR well moulding plate

1. **Either**

(a) Immerse the gel/plate in a solution of EtBr in water or electrophoresis buffer, 0.5 µg/mL for 15-30 min⁷ OR 0.1 µg/mL for 60 min.

Or

(b) Make a staining vessel from the well moulding plate using the thicker silicone rubber cord and clamp to the gel plate (see item 7). Pour EtBr solution, 0.5 µg/mL, into the gap and leave for 1 hour.

2. Photograph on the transilluminator **gel side down**.

Notes:

⁶From 10 mg/mL stock: 0.5 µg/mL is 1:20,000 dilution = 5 µL/100 mL; 0.1 µg/mL is 1:100,000 = 1 µL/100 mL

⁷Destaining for an hour or more in water or buffer can reduce high or uneven background

7. The 'KT Polyacrylamide Gel Staining System':

WHAT YOU NEED
White silicone cord, 2.62 mm diameter
Well moulding plate used to cast gel
Clamps or fold-back clips

- Use the gel moulding piece to make a staining 'cell'
 - Make a seal with the 2.62 mm diameter cord in the same way as you used the 2 mm cord to cast the gel
 - Clamp the gel plate to the seal with clips
- Apply the appropriate volume of stain in the gap between the gel and the glass
 - It should be about half the volume you used to make the gel
 - We recommend EtBr at 0.5 µg/mL for 15 to 30 min.
- Pour off the EtBr solution and dispose of it according to your institute's regulations (see notes on Ethidium Bromide disposal — page 7)
- Separate the plate with the gel from the mould
- Lay **gel-down** on the UV transilluminator
- Switch the illuminator to full power
- If the background is high, destain the gel in TBE for 1 hour

Notes:

This protocol reduces the volume and amount of EtBr to a minimum, alleviating the problem of disposal: the concentration of EtBr remaining is lower than 0.5 µg/mL, the level that most regard as safe to dispose of without special precautions. (See page 7)