

DNA ELECTROPHORESIS

TBE buffer 10x TBE (1 liter):

108 g Tris

55 g Boric acid in 900 ml distilled water.

Add 40 ml 0.5 M Na₂EDTA (pH 8.0)

Adjust volume to 1 Liter.

Store at room temperature.

Note: 10x TBE may take some time to dissolve, even with fast stirring

TBE can be diluted to 1X prior to use in electrophoresis, 0.5x is acceptable as well.

20% acrylamide (7 M urea) solution for gel

210.2 g urea

250 ml 40% acrylamide/bis solution

25 ml 10X TBE buffer

Mix and adjust volume to 500 ml

10% APS

1 g APS

9 ml H₂O

Mix and adjust volume to 10 ml

Gel

7 ml 20% acrylamide (7M) solution

70 µL 10% APS

7 µL TEMED

Hardening time – 10 min

Running conditions:

- 1) 10 min at 300 V
- 2) Load DNA
- 3) 5 min at 150 V
- 4) 1h at 300 V

Loading Buffer:

720 mg urea

60 µL 0.5 M EDTA or 600µL 50 mM EDTA

3 µL 1M Tris buffer (pH 7.4)

Adjust total volume to 1.5 ml