

1. Preparation of the agarose gel solution:

WHAT YOU NEED
LE Seakem Agarose
10X TBE or TAE buffer
Ethidium bromide solution
Microwave

1. Choose a glass bottle or conical flask that is 2-4 times the volume of the solution because agarose boils over easily.
2. Sprinkle pre-measured agarose powder onto room temperature 1X buffer and swirl gently to mix. Weigh the bottle or flask to note the initial weight.
3. Cover with microwaveable plastic wrap and pierce.
4. Heat the container on HIGH power for about 45 seconds or until bubbles appear. ****Caution**** The solution can become superheated and boil over on your hands. As you remove the container, give it a gentle swirl ****Wear Gloves**** and ****Hold at arms length****
5. Reheat on HIGH power until the solution comes to the boil. Remove, gently swirl and check to see that all the particles are dissolved and the solution is mixed thoroughly.
6. Check the final weight is the same as the initial volume – add distilled water to make up losses.

2. Pouring the gel:

WHAT YOU NEED
Galileo horizontal electrophoresis unit
Triple Dye Loading solution
50 bp DNA ladder
10X TBE or TAE buffer
Power pack

1. Allow the agarose solution to cool to 50-60°C. If an 'in-gel' staining method is to be used, add the nucleic acid stain and swirl gently to mix thoroughly.
2. Three sizes of the Galileo horizontal gel electrophoresis units have been supplied. To cast a gel, the trays do **not** need to be taped. For the 7 x 8 cm and 12 x 14 cm units (RapidCast system), the tray end gaskets form a seat against the buffer chamber walls when the tray is placed cross-ways. In the 23 x 25 cm unit (ExpressCast system), gasketed end gates fit in grooves at the tray ends to seal them for gel pouring.
3. Gel thickness has a profound effect on the resolution of smaller fragments – for optimal resolution cast horizontal gels **3-4mm thick**.
4. After pouring gel leave to set for 1 hour with the gel lid on if possible.

3. Loading and running the gel:

1. Add 1 μ L of triple dye loading buffer¹ per 5 μ L of sample and mix thoroughly.
2. DNA ladders (50 bp² and 100 bp³) are provided ready-to-use in loading dye containing 10mM EDTA, 10% glycerol, 0.015% bromophenol blue and 0.17% SDS. For best results load 10 μ L per well

Notes:

¹Triple dye DNA loading buffer is supplied in a 1.2 mL vial containing 50% (w/v) sucrose and 40mM tris base in distilled, deionized water. It is a non-denaturing loading buffer for native polyacrylamide and agarose gel applications and contains 3 tracking dyes (Bromophenol Blue, Xylene Cyanol, and Orange G) at 6X concentration.

²The 50bp ladder contains 14 discrete fragments ranging from 25 bp to 650 bp in 50 bp increments with double intensity reference bands at 200 bp and 400 bp.

³The 100bp ladder contains 11 discrete fragments ranging from 50bp to 1,000 bp, with a high intensity reference band at 500 bp.

3. Loading and running the gel (Continued):

3. Buffer Depth should be 3-5mm. TBE is preferred for separation of DNA <1Kb.

Gel length for calculating voltage gradient:

- 0708 Galileo unit = 8.0 cm
- 1214 Galileo unit = 13.5 cm
- 2325 Galileo unit = 24.5 cm

For <1 Kb voltage should be 5 V/cm

Gel should be run until the band of interest has migrated 40-60% down the length of the gel.

4. Photographing the gel:

See Gel Photography Protocol — page 6.

Notes:

Power Supply: Voltage which is too high will cause band streaking and smearing, if the voltage is too low, it will cause band broadening due to diffusion.