ALKALINE AGAROSE GELS

Alkaline agarose gels (McDonnell et al. 1977) are used: (1) to analyze the size of the DNA strand in nuclease-S1-resistant DNA ·RNA hybrids (Favaloro et al. 1980); (2) to check the size of first and second DNA strands synthesized by reverse transcriptase; (3) to check for nicking activity in enzyme preparations used for molecular cloning; (4) to calibrate the reagents used in nick translation of DNA.

Because addition of sodium hydroxide to hot agarose solution causes hydrolysis of the polymer, the gels are prepared in a neutral, unbuffered solution (50 mM NaCl and 1 mM EDTA) and equilibrated in alkaline electrophoresis buffer before running.

1. Add the correct amount of powdered agarose to a measured quantity of 50 mM NaCl and 1 mM EDTA.

2. Heat the slurry in a boiling-water bath or in a microwave oven until the agarose dissolves.

3. Cool the solution to 50°C, pour the gel and mount it in the electrophoresis tank.

4. Add sufficient alkaline electrophoresis buffer to cover the gel to a depth of 3-5 mm.

   Alkaline electrophoresis buffer:

   30 mM Na0H
   1 mM EDTA

   Allow the buffer to soak into the gel for at least 30 minutes before loading the samples of DNA.

5. Samples of DNA to be analyzed on alkaline agarose gels are precipitated with ethanol and dissolved in 10-20 ul of alkaline loading buffer.

   Alkaline loading buffer:

   50 mM Na0H
   1 mM EDTA
   2.5% Ficoll (type 400; Pharmacia)
   0.025% bromocresol green

6. Before loading, remove the excess alkaline electrophoresis buffer from above the surface of gel. Sufficient buffer should remain to cover the gel to a depth of 1 mm.

7. Load the DNA samples. Electrophoresis is carried out at voltages up to 7.5 V/cm until the dye has migrated approximately 8 cm (about 15 V-hr/cm).

Because bromocresol green diffuses rapidly out of alkaline gels into the electrophoresis buffer, a second glass plate should be placed directly on top of the gel after the dye has migrated out of the loading slot.

8. In many cases, DNA analyzed by alkaline gel electrophoresis is labeled with ^{32}P, which can be detected by autoradiography. At the end of the run, the gel is removed from the tank and soaked for 30 minutes at room temperature in 7% trichloracetic acid. The gel is then mounted on a glass plate and dried for several hours under many layers of Whatman 3MM paper weighted with another glass plate. The dried gel is then covered with Saran Wrap and autoradiographed at room temperature or at -70°C with an intensifying screen.

   If the DNA is unlabeled, the gel should be soaked for 1 hour in neutralizing solution (1 M Tris ·Cl [pH 7.6] and 1.5 M NaCl). The DNA can then be transferred to a nitrocellulose filter and detected by hybridization to an appropriate ^{32}P-labeled probe.

References: Procedure from Maniatis cloning manual
1. McDonnell, M.W., M.N. Simon and F.W. Studier. 1977. J. Mol. Biol. **110**, 119.
2. Favaloro, J., R. Treisman and R. Kamen. 1980. Methods Enzymol.**65**, 718.