

# Procedures for Gel Preparation with SequaGel® XR Concentrate



SequaGel XR Concentrate (EC-843) is a proprietary solution of acrylamide and acrylamide derivatives in distilled, deionized water. Store tightly capped in a dark area at room temperature (20°C). Acrylamide has been found to be neurotoxic. Protective gloves and eyewear should be worn while handling these products. If accidental exposure occurs, contact a physician immediately.

## Mix SequaGel System Components

| % Monomer | SequaGel XR Concentrate | 10X TBE                     | Urea                     | Deionized Water   | TEMED | 10% APS |
|-----------|-------------------------|-----------------------------|--------------------------|-------------------|-------|---------|
| 5         | 10 ml                   | 6 ml (0.6X)<br>10 ml (1.0X) | 36 g (6 M)<br>42 g (7 M) | Q.S. to<br>100 mL | 50 µl | 500 µl  |
| 6         | 12 ml                   | 6 ml (0.6X)<br>10 ml (1.0X) | 36 g (6 M)<br>42 g (7 M) | Q.S. to<br>100 mL | 50 µl | 500 µl  |
| 8         | 16 ml                   | 6 ml (0.6X)<br>10 ml (1.0X) | 36 g (6 M)<br>42 g (7 M) | Q.S. to<br>100 mL | 50 µl | 500 µl  |

Prepare the gel solution according to Table 1 above. Add the first four components to a thick-walled Erlenmeyer flask and swirl to dissolve the urea. Warm if necessary but bring

to room temperature before polymerization. If desired, filter the solution through Whatman® #1 filter paper or a ≤0.45 µm cellulose acetate filter.

## Add Initiators and Cast Gel

Add TEMED (See Table 1). Swirl gently to mix. Add FRESHLY PREPARED 10% Ammonium Persulfate (See Table 1). Swirl gently to mix. Cast the gel. Insert the comb and allow to polymerize one to two hours. NOTE: After two hours of

polymerization wrap each end of the gel cassette with clear plastic wrap. This is important to keep the ends of the gel from drying and to maintain sample well integrity. Appropriately wrapped gels may be stored for up to 48 hours.

## Suggestions for Best Results

- SequaGel XR Concentrate is a 50% solution. A 5-6% gel is generally recommended. Use 1X TBE for maximum base read and/or multiple loads. Use 0.6X TBE for fast runs. Use an 8% gel in 0.6X TBE to read DNA sequence close to the primer.
- Clean glass plates thoroughly. Rinse with ethanol and wipe dry. Apply Glass Free (Cat. #EC-621) to one plate to ensure release after electrophoresis.
- For the most consistent results, degas the gel solution prior to adding initiators. Add a stirring bar to the solution and stopper the flask. Degas the solution under vacuum for 5 minutes while stirring on a magnetic stirrer. After deaeration, warm in a 30°C bath if necessary to redissolve the urea.
- Prerun the gel for 15-30 minutes before loading the samples. For small (35ml, 20 X 40 X 0.04 cm) gels use 30-35 Watts. For large (70ml, 40 X 40 X 0.04 cm) gels use 55-65 Watts. The gel temperature should be between 45-50°C.

● Use Table 2 below to monitor electrophoresis progress by means of the co-migration of the bromophenol blue and xylene cyanole tracking dyes with your samples. When doing multiple loads, the next load should be added when the bromophenol blue is 3-4 cm from the bottom of the gel.

| Gel % | Bromophenol Blue (nucleotides) | Xylene Cyanole (nucleotides) |
|-------|--------------------------------|------------------------------|
| 5     | 35                             | 130                          |
| 6     | 26                             | 106                          |
| 8     | 19                             | 75                           |

- After the completion of the run, allow the plates to cool 10-15 minutes before separation.

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