

SequaGel[®]

**national
diagnostics**

Procedures for Gel Preparation with SequaGel 4, 4.25, 4.75, 6, 8

SequaGel 4, 4.25, 4.75, 6 and 8 (EC-834, EC-832, EC-837, EC-836 or EC-838) are convenient two solution systems. The SequaGel Monomer Solution contains Urea as well as Acrylamide and Bis-acrylamide (19:1, w/w) in deionized, distilled water. The SequaGel Complete Buffer Solution contains 5X TBE and TEMED in deionized, distilled water. Store solutions tightly capped in a dark area at room temperature (20°C). If SequaGel Monomer Solution is refrigerated, urea may precipitate. This urea will redissolve when the solution returns to room temperature. *Acrylamide has been found to be neurotoxic. Protective eyewear and gloves should be worn while handling these products. If accidental exposure occurs, contact a physician immediately.*

Mix SequaGel Monomer Solution and Buffer

Add appropriate volumes of SequaGel Monomer Solution and SequaGel Complete Buffer to a thick-walled Erlenmeyer flask (see Table 1). If desired, the solution may be degassed by stirring under vacuum for two minutes. Bring to room temperature before polymerization.

Table 1: Volumes of SequaGel 4, 4.25, 4.75, 6 or 8 and SequaGel Complete Buffer to prepare 100mL gel solution

SequaGel Monomer Solution	SequaGel Complete Buffer	10% Ammonium Persulfate
80 mL	20 mL	800 µL

Add APS and Cast Gel

Add FRESHLY PREPARED 10% Ammonium Persulfate (see Table 1). Swirl gently to mix, and 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

- Clean glass plates thoroughly. Rinse with ethanol and wipe dry. Apply Glass Free (Cat. #EC-621) to one plate to ensure release after electrophoresis.
- Prerun the gel for 15-30 minutes before loading the samples. The gel temperature should be between 45-50°C.
- After the completion of the run, allow the plates to cool 10-15 minutes before separation.
- Degassing the casting solution prior to initiation will improve reproducibility.

Table 2: Tracking Dye Migration in SequaGel Solutions

Gel %	Bromophenol Blue (nucleotides)	Xylene Cyanole (nucleotides)
4	36	155
4.25	34	148
4.75	31	136
6	26	110
8	20	75

Use the table above to monitor electrophoresis progress by means of dye-migration. When doing multiple loads, the next load should be added when the bromophenol blue is 3-4 cm from the bottom of the gel.

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10X TBE

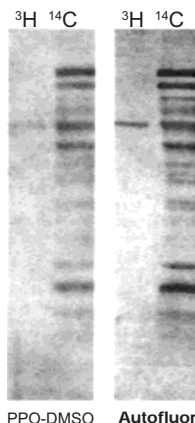
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