

Procedures for Gel Preparation with the SequaGel® Sequencing System

SequaGel Sequencing System Kit (EC-833) contains SequaGel Concentrate, SequaGel Diluent, and SequaGel Buffer. Liter bottles of SequaGel Concentrate contain 237.5 grams of Acrylamide, 12.5 grams of methylene bisacrylamide, and urea in a deionized aqueous solution. SequaGel Diluent is supplied in 450 ml and 1 liter bottles containing urea in deionized water. SequaGel Buffer is supplied in 100 ml and 200 ml bottles containing 0.89M Tris-Borate-20mM EDTA buffer pH 8.3 (10X TBE) and urea. Store solutions tightly capped in a dark area at room temperature (20°C). Urea may precipitate if these solutions are refrigerated. This urea will redissolve when the solution returns to room temperature. *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

Table 1: Formulations for Commonly Used Gel Percentages
100 ml Gel Casting Solution

DNA Fragment Size (in nucleotides)	% Monomer	SequaGel Concentrate	SequaGel Diluent	SequaGel Buffer
>200	4	16 ml	74 ml	10 ml
80-200	5	20 ml	70 ml	10 ml
60-150	6	24 ml	66 ml	10 ml
40-100	8	32 ml	58 ml	10 ml
10-50	12	48 ml	42 ml	10 ml
<20	20	80 ml	10 ml	10 ml

Determine how much SequaGel Concentrate, Diluent, and Buffer you need to make your gels using either Table 1

$$V_c = \frac{(V_t)(X)}{25}$$

$$V_b = 0.1 (V_t)$$

$$V_d = V_t - (V_c + V_b)$$

V_c = SequaGel Concentrate Volume

V_b = SequaGel Buffer Volume

V_d = SequaGel Diluent Volume

V_t = Total Casting Solution Volume

X = % Gel Desired

above or use the formulas below. Combine the necessary components in an Erlenmeyer flask. Swirl gently to mix.

EXAMPLE: To make 100ml of an 8% sequencing gel, calculate the SequaGel solution volumes to be added as follows:

$$V_c = \frac{(100)(8)}{25} = 32\text{ml SequaGel Concentrate}$$

$$V_b = 0.1 (100) = 10\text{ml SequaGel Buffer}$$

$$V_d = 100 - (32 + 10) = 58\text{ml SequaGel Diluent}$$

Add Initiators and Cast Gel

Add 40 microliters of TEMED for every 100 ml of gel casting solution. Swirl gently to mix. Add 0.8 ml of FRESHLY PREPARED 10% Ammonium Persulfate for every 100ml of gel casting solution. 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

- Clean glass plates thoroughly. Rinse with ethanol and wipe dry. Apply Glass Free (Cat. #EC-621) to one plate to ensure release after electrophoresis.
- Degassing the casting solution prior to initiation will improve reproducibility.
- 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.

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.