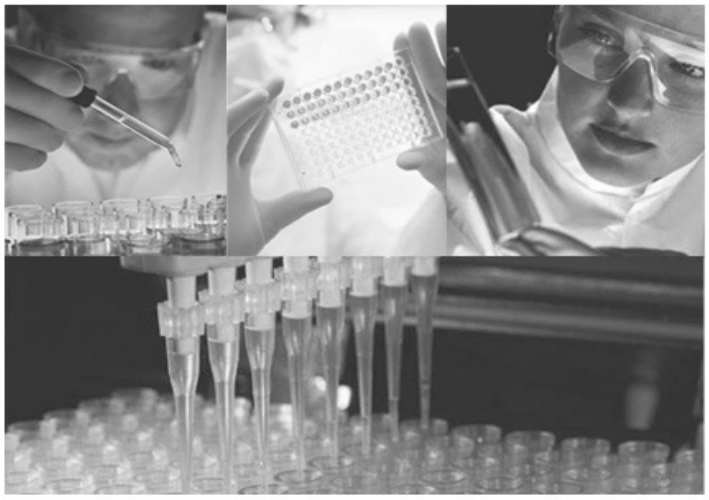
national diagnostics



SequaGel® UreaGel™ System

Ready-to-Use System for Pouring 19:1 Acrylamide:Bisacrylamide Denaturing Gels from 4% to 20% Monomer

The SequaGel UreaGel System (EC-833) contains UreaGel Concentrate, UreaGel Diluent and UreaGel Buffer. UreaGel Concentrate contains 237.5 g/L of acrylamide, 12.5 g/L of methylene bisacrylamide, and 7.5M urea in a deionized aqueous solution. UreaGel Diluent is supplied in 450 ml and 1 liter bottles containing urea in deionized water. UreaGel 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.

DANGER: *Acrylamide has been found to be neurotoxic. Protective gloves and eyeware should be worn while handling these products. If accidental exposure occurs, contact a physician immediately.*

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.

Mix UreaGel System Components

Formulations for Commonly Used Gel Percentages
100 ml Gel Casting Solution

DNA Fragment Size (in nucleotides)	% Monomer	UreaGel Concentrate	UreaGel Diluent	UreaGel 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 UreaGel Concentrate, Diluent, and Buffer you need to make your gels using either Table 1 above or use the formulas below. Combine the necessary components in an Erlenmeyer flask. Swirl gently to mix.

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

$$V_b = 0.1\ (V_t)$$

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

V_c = UreaGel Concentrate Volume
V_b = UreaGel Buffer Volume
V_d = UreaGel Diluent Volume
V_t = Total Casting Solution Volume
X = % Gel Desired

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

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

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

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

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.

Tracking Dye Migration

Tracking Dye Migration in UreaGel Solutions

Gel %	Bromophenol Blue (nucleotides)	Xylene Cyanole (nucleotides)
4	30	155
6	25	110
8	20	75
10	10	55
12	8	45

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.