



# ProtoGel<sup>®</sup> 30%

## Procedures for Gel Preparation Using ProtoGel

- 37.5:1 Acrylamide:Bisacrylamide Stabilized Solution
- Optimized for SDS-PAGE (Laemmli gels) of Proteins
- Consistently Crystal Clear Gels, Zero Fluorescence
- Stabilized for Long Shelf Life

### DETERMINING GEL FORMULATION

Use the chart below to determine the volumes of reagents required for desired gel composition. If the percentage gel which you are running is not included in the table, use the formula below to calculate the volumes of ProtoGel, ProtoGel Resolving Buffer, and other reagents needed.

Volumes of 30% ProtoGel and ProtoGel Resolving Buffer To Achieve Common Gel Percentages					
% Monomer	METHOD 1		OR	METHOD 2	
	Volume of ProtoGel, and Resolving Buffer to use			Volume of ProtoGel and reagents to use	
6%	ProtoGel:	20.0ml	1.5 M Tris-HCl, pH 8.8:	ProtoGel:	20.0ml
	Resolving Buffer:	25.0ml		25.0ml	
	Deionized H <sub>2</sub> O:	53.9ml		10% SDS: 1.0ml	
8%	ProtoGel:	26.7ml		Deionized H <sub>2</sub> O:	52.9ml
	Resolving Buffer:	25.0ml		ProtoGel:	26.7ml
	Deionized H <sub>2</sub> O:	47.2ml	25.0ml		
10%	ProtoGel:	33.3ml	10% SDS:	1.0ml	
	Resolving Buffer:	25.0ml	Deionized H <sub>2</sub> O:	39.6ml	
	Deionized H <sub>2</sub> O:	40.6ml	ProtoGel:	33.3ml	
12%	ProtoGel:	40.0ml	25.0ml	25.0ml	
	Resolving Buffer:	25.0ml	10% SDS:	1.0ml	
	Deionized H <sub>2</sub> O:	33.9ml	Deionized H <sub>2</sub> O:	32.9ml	
15%	ProtoGel:	50.0ml	ProtoGel:	50.0ml	
	Resolving Buffer:	25.0ml	25.0ml		
	Deionized H <sub>2</sub> O:	23.9ml	10% SDS: 1.0ml		
			Deionized H <sub>2</sub> O:	22.9ml	

Note: The amount of ProtoGel Resolving Buffer used is always the same, regardless of percentage of monomer in the gel (25.0ml of ProtoGel resolving Buffer per 100ml of gel casting solution).

### DETERMINING GEL FORMATION, CONT'D

The volume of ProtoGel required for gel casting solutions of any volume and acrylamide concentration may be calculated from the following formula:

$$V_p = \frac{(X)(V_t)}{30}$$

where,  $V_p$  = Volume of 30% ProtoGel  
 $X$  = % Monomer Desired in Gel  
 $V_t$  = Total Volume of Gel Casting Solution

EXAMPLE: To make 100 ml of a 10% monomer gel, calculate the volume of Protogel to add as follows:

$$V_p = \frac{(10)(100)}{30} = 33.3 \text{ ml}$$

### DE-GAS GEL

In most cases ProtoGel will gel without de-gassing. However, de-gassing will provide the highest quality, most consistent gels. If de-gassing is desired, use the following procedure: Add a stirring bar to the solution and stopper the flask. De-gas the solution under vacuum for 5 minutes while stirring with a magnetic stirrer.

### ADD INITIATORS AND CAST GEL

Add 1.0ml of 10% (w/v) ammonium persulfate for every 100ml of gel casting solution. Swirl gently to mix. Add 0.1 ml of TEMED for every 100ml of gel casting solution. Swirl gently to mix. Pour the solution into the gel casting cassette. The gel should begin to set in 10-20 minutes. To provide a sharp interface, overlay the gel with water saturated n-butanol during polymerization. Flush butanol away with water just before casting the stacking gel (below).

### POUR STACKING GEL

Use ProtoGel Stacking Buffer to make 10ml of a 4% stacking gel:

ProtoGel: 1.3ml  
 ProtoGel Stacking Buffer: 2.5ml  
 Deionized Water: 6.1ml

Add 0.05ml 10% ammonium Persulfate and 0.01 ml of TEMED. Gel will begin to set in 20 minutes.

NOTE: A solution of 0.5M Tris-HCl, 0.4% SDS, pH 6.8 may be substituted for ProtoGel Stacking Buffer.

### FOR ADDITIONAL INFORMATION AND ORDER PLACEMENT:

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