

Procedures for Gel Preparation with ProtoGel

Determine Gel Formulation

ProtoGel allows you to prepare gels of any percentage monomer desired. Use the chart at right to determine the volumes of reagents required for the 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 Buffer, and other reagents needed.

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 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.

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

Volumes of ProtoGel and ProtoGel Buffer to Achieve Common Gel Percentages

% Monomer	METHOD 1	OR	METHOD 2
	Volume of ProtoGel, ProtoGel Buffer to use		Volume of ProtoGel, and reagents to use
6%	ProtoGel: 20.0ml ProtoGel Buffer: 26.0ml Deionized H ₂ O: 52.9ml		ProtoGel: 20.0ml 1.5 M Tris-HCl: 25.0ml 10% SDS: 1.0ml Deionized H ₂ O: 52.9ml
	ProtoGel: 26.7ml ProtoGel Buffer: 26.0ml Deionized H ₂ O: 46.2ml		ProtoGel: 26.7ml 1.5 M Tris-HCl: 25.0ml 10% SDS: 1.0ml Deionized H ₂ O: 46.2ml
10%	ProtoGel: 33.3ml ProtoGel Buffer: 26.0ml Deionized H ₂ O: 39.6ml		ProtoGel: 33.3ml 1.5 M Tris-HCl: 25.0ml 10% SDS: 1.0ml Deionized H ₂ O: 39.6ml
	ProtoGel: 40.0ml ProtoGel Buffer: 26.0ml Deionized H ₂ O: 32.9ml		ProtoGel: 40.0ml 1.5 M Tris-HCl: 25.0ml 10% SDS: 1.0ml Deionized H ₂ O: 32.9ml
15%	ProtoGel: 50.0ml ProtoGel Buffer: 26.0ml Deionized H ₂ O: 22.9ml		ProtoGel: 50.0ml 1.5 M Tris-HCl: 25.0ml 10% SDS: 1.0ml Deionized H ₂ O: 22.9ml

Add 1.0 ml 10% (w/v) Ammonium Persulfate and 0.10 ml TEMED to result in 100 ml of gel casting solution.

NOTE: The amount of ProtoGel buffer used is always the same, regardless of percentage of monomer in the gel (26.0ml of ProtoGel Buffer per 100ml of gel casting solution).

De-gas Gel

In most cases ProtoGel will gel without de-gassing. However, 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 0.1ml 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.

Pour Stacking Gel

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

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

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

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

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