ProtoGel® 30%

- **37.5:1 Acrylamide : Bisacrylamide**
- **Ultra-Pure, Stabilized Solution**

Method for SDS-PAGE

1 Gel Formulation - Laemmli SDS-PAGE

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 Solution Components for Common Gel Percentages
Using Premixed 4X Resolving Buffer or 1.5 M Tris-HCl

Gel %			- or -	
6%	ProtoGel 30%:	20.0ml	ProtoGel 30%:	20.0ml
	4X Resolving Buffer:	25.0ml	1.5 M Tris-HCl, pH 8.8:	25.0ml
	Deionized H_2O:	53.9ml	10% SDS:	1.0ml
			Deionized H_2O:	52.9ml
8%	ProtoGel 30%:	26.7ml	ProtoGel 30%:	26.7ml
	4X Resolving Buffer:	25.0ml	1.5 M Tris-HCl, pH 8.8:	25.0ml
	Deionized H_2O:	47.2ml	10% SDS:	1.0ml
			Deionized H_2O:	46.2ml
10%	ProtoGel 30%:	33.3ml	ProtoGel 30%:	33.3ml
	4X Resolving Buffer:	25.0ml	1.5 M Tris-HCl, pH 8.8:	25.0ml
	Deionized H_2O:	40.6ml	10% SDS:	1.0ml
			Deionized H_2O:	39.6ml
12%	ProtoGel 30%:	40.0ml	ProtoGel 30%:	40.0ml
	4X Resolving Buffer:	25.0ml	1.5 M Tris-HCl, pH 8.8:	25.0ml
	Deionized H_2O:	33.9ml	10% SDS:	1.0ml
			Deionized H_2O:	32.9ml
15%	ProtoGel 30%:	50.0ml	ProtoGel 30%:	50.0ml
	4X Resolving Buffer:	25.0ml	1.5 M Tris-HCl, pH 8.8:	25.0ml
	Deionized H_2O:	23.9ml	10% SDS:	1.0ml
			Deionized H_2O:	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).

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:

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

2 Add Initiators and Cast Gel

For optimal results degas gel solution for 10 minutes under vacuum aspiration prior to innitiation with APS and TEMED. 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).

3 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 0.01ml 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.

4 Select Running Buffer

Laemmli SDS-PAGE - 1X Tris-Glycine SDS is the most suitable tank buffer for most SDS-PAGE applications: Order # EC-870.

Small Protein SDS-PAGE (< 20kD) - National Diagnostics unique Tris-Tricine-SDS running buffer helps resolve smaller proteins without requiring the full Schagger - Von Jagow protocol: Order # EC-869.

ProtoGel Safety Information

	CAS #
Acrylamide	79-06-1
Bis-Acrylamide	110-26-9

R: 45-46-24/25-48/23/24/25
S: 53-45

Risk – May cause cancer. May cause heritable genetic damage. Also toxic in contact with skin and if swallowed. Danger of serious damage to health by prolonged exposure through inhalation, in contact with skin or if swallowed.

Safety – Avoid exposure, obtain special instructions before use. In case of accident or if you feel ill, seek medical advice immediately (show the label where possible).

$ Order Numbers for this Protocol

ProtoGel 30% - EC-890
4X Resolving Buffer - EC-892
4X Stacking Buffer - EC-893
10X Tris Glycine SDS - EC-870
10X Tris Tricine SDS EC-869
Protein Loading Buffer Blue 2X EC-886
Ammonium Persulfate - EC-504
TEMED - EC-503

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usa: 1-800-526-3867 europe: 44(0) 1482 646020 www.nationaldiagnostics.com