ProtoGel® 30%

national diagnostics

- *37.5:1 Acrylamide:Bisacrylamide*
- *Ultra-Pure, Stabilized Solution*
- *Consistently Crystal Clear Gels*
- *Zero Fluorescence*

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 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 (mL)

Using Premixed 4X Resolving Buffer or 1.5M Tris-HCl

Gel %			-OR-	
6	ProtoGel 30%	20.0	ProtoGel 30%	20.0
	4X Resolving Buffer	25.0	1.5M Tris-HCl (pH8.8)	25.0
	Deionized Water	53.9	10% SDS	1.0
			Deionized Water	52.9
8	ProtoGel 30%	26.7	ProtoGel 30%	26.7
	4X Resolving Buffer	25.0	1.5M Tris-HCl (pH8.8)	25.0
	Deionized Water	47.2	10% SDS	1.0
			Deionized Water	46.2
10	ProtoGel 30%	33.3	ProtoGel 30%	33.3
	4X Resolving Buffer	25.0	1.5M Tris-HCl (pH8.8)	25.0
	Deionized Water	40.6	10% SDS	1.0
			Deionized Water	39.6
12	ProtoGel 30%	40.0	ProtoGel 30%	40.0
	4X Resolving Buffer	25.0	1.5M Tris-HCl (pH8.8)	25.0
	Deionized Water	33.9	10% SDS	1.0
			Deionized Water	32.9
15	ProtoGel 30%	50.0	ProtoGel 30%	50.0
	4X Resolving Buffer	25.0	1.5M Tris-HCl (pH8.8)	25.0
	Deionized Water	23.9	10% SDS	1.0
			Deionized Water	22.9

Note: The amount of ProtoGel Resolving Buffer is always the same, regardless of percentage of monomer in the gel (25mL of ProtoGel Resolving Buffer per 100mL gel casting solution).

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

$$V_p = (XV_t) / 30$$

where V_p = volume of 30% ProtoGel, X = percent monomer desired in gel and V_t = total volume of gel casting solution.

EXAMPLE: To make 100 mL of a 10% percent monomer gel, the amount of ProtoGel to add is calculated as follows:

$$V_p = (10 * 100) / 30 = 33.3$$

2. Add Initiators and Cast Gel

For optimal results, degas gel solution for 10 minutes under vacuum aspiration prior to initiation with ammonium persulfate and TEMED. Add 1.0 mL of 10% (w/v) ammonium persulfate for every 100 mL of gel casting solution. Swirl gently to mix. Add 0.1 mL of TEMED for every 100 mL of gel casting solution. 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 10 mL of a 4% stacking gel as follows:

ProtoGel:	1.33 mL
ProtoGel Stacking Buffer:	2.50 mL
Deionized Water:	6.11 mL

Afterwards, add 0.05 mL 10% ammonium persulfate and 0.01 mL TEMED. Gel will begin to set in 20 minutes.

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

4. Select Running Buffer

Laemmli SDS-PAGE - 1X Tris-Glycine-SDS (EC-870) is the most sutiable tank buffer for most SDS-PAGE applications.

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

Order Numbers for this Protocol

ProtoGel 30% Order No. EC-890	450 mL 1 liter
4X Resolving Buffer Order No. EC-892	450 mL 1 liter
4X Stacking Buffer Order No. EC-893	200 mL
10X Tris-Glycine-SDS Bufffer Order No. EC-870	1 liter 4 liter
10X Tris-Tricine-SDS Buffer Order No. EC-869	1 liter
2X Protein Loading Buffer Blue Order No. EC-886	10 X 1 mL
5X Protein Loading Buffer Order No. EC-887	10 X 1mL
Ammonium Persulfate Order No. EC-504	25 g 100 g
TEMED Order No. EC-503	25 mL

For Additional Information & Order Placement:

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