

Procedures for Gel Preparation with AccuGel™ 19:1 and AccuGel™ 29:1 for DNA and RNA

AccuGel 19:1
Order No. EC-850

450 mL
1 Liter

AccuGel 29:1
Order No. EC-852

450 mL
1 Liter

Electrophoresis gels for nucleic acids are commonly cast in the range of 4% to 20% monomer. The acrylamide percentage to be used depends on the size of the nucleic acid fragments to be fractionated. The greater the number of base pairs to be separated, the larger the pore size required, and therefore the lower the acrylamide percentage to be used. For the electrophoresis of single stranded DNA or RNA, typically AccuGel 19:1 is used to formulate denaturing gels containing urea. AccuGel 29:1 is typically used to formulate native gels, which do not contain urea, for the electrophoresis of double stranded nucleic acid samples.

DNA and Dye Comigration Tables

Denaturing AccuGel 19:1 Gels

Gel %	Size Range (bp)	Bromophenol Blue (nucleotides)	Xylene Cyanol (nucleotides)
4	>250	30	155
6	60-250	25	110
8	40-120	20	75
10	20-60	10	55
12	10-50	8	45

Native AccuGel 29:1 Gels

Gel %	Size Range (bp)	Bromophenol Blue (nucleotides)	Xylene Cyanol (nucleotides)
4	1000-2000	95	450
6	70-450	60	240
8	60-400	45	160
10	50-300	35	120
12	40-200	20	70

Mix Gel Solution

Calculate how much AccuGel you need to make your gels by using the table at left or the formulas below. Bring up to the desired final volume with your usual buffers and distilled water. Pour the solution into an Erlenmeyer flask with a side-arm. In most cases, AccuGel will gel without degassing. However, for optimum reproducibility, add a stirring bar to the solution and stopper the flask. Degas the solution under a vacuum for 5 minutes while stirring on a magnetic stirrer.

$$V_{A30} = \frac{(X)(V_1)}{30}$$

$$V_{A40} = \frac{(X)(V_1)}{40}$$

$$V_{A30} = \text{Volume of 30\% AccuGel to be used (ml)} \quad \text{OR} \quad V_{A40} = \text{Volume of 40\% AccuGel to be used (ml)}$$

$$X = \% \text{ gel desired} \quad V_1 = \text{Total volume of gel casting (solution desired (ml))}$$

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

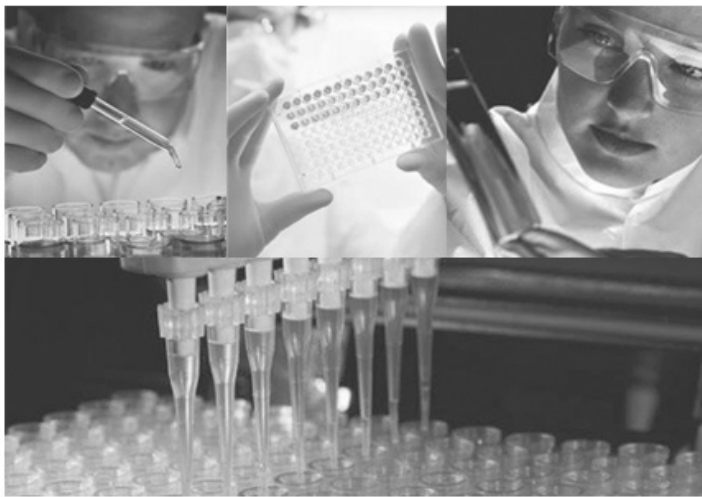
Add APS and Cast Gel

Add 1.0ml of 10% (w/v) FRESHLY PREPARED Ammonium Persulfate for every 100ml of gel casting solution. Swirl gently to mix. Add 100 microliters 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. Polymerization should be permitted to continue for a minimum of 1.5-2 hours before gel is run. 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.

AccuGel Formulations for Commonly Used Gel Percentages

100 ml Gel Casting Solution

	4%	4.25%	4.75%	5%	6%	8%	10%	12%
30% AccuGel (ml)	13.3	14.1	15.8	16.7	20	26.7	33.3	40
40% AccuGel (ml)	10	10.6	11.9	12.5	15	20	25	30
Urea(g)	6M	36	36	36	36	36	36	36
	7M	42	42	42	42	42	42	42
	8M	48	48	48	48	48	48	48
10X TBE (ml)	0.6X	6	6	6	6	6	6	6
	1.0X	10	10	10	10	10	10	10
Distilled Water	QS to 100 ml	QS to 100 ml	QS to 100 ml	QS to 100 ml	QS to 100 ml	QS to 100 ml	QS to 100 ml	QS to 100 ml



Procedures for Gel Preparation with AccuGel™ 29:1 with SDS-PAGE

AccuGel 29:1
Order No. EC-852

450 mL
1 Liter

AccuGel 29:1 allows you to prepare gels of any percentage monomer desired. Use the chart below 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 AccuGel 29:1 30%, ProtoGel Resolving Buffer, and other reagents needed.

Determine Gel Formulation

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

$$V_p = \frac{(X) (V_t)}{30^*} \quad \text{*Use '40' in this equation for AccuGel 29:1 40%}$$

where, V_p = Volume of AccuGel 29:1 30%
 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 AccuGel 29:1 30% to add as follows:

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

DE-GAS GEL

In most cases AccuGel 29:1 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 0.1 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.

Volumes of AccuGel 29:1 30% and ProtoGel Resolving Buffer To Achieve Common Gel Percentages

% Monomer	METHOD 1		OR	METHOD 2						
	Volume of AccuGel and Resolving Buffer to use			Volume of AccuGel and reagents to use						
6%	AccuGel 29:1 30%:	20.0ml	AccuGel 29:1 30%:	20.0ml	1.5 M Tris-HCl, pH 8.8:	25.0ml	10% SDS:	1.0ml	Deionized H ₂ O:	52.9ml
	4X Resolving Buffer:	25.0ml		1.5 M Tris-HCl, pH 8.8:		25.0ml				
	Deionized H ₂ O:	53.9ml		10% SDS:		1.0ml				
8%	AccuGel 29:1 30%:	26.7ml	AccuGel 29:1 30%:	26.7ml	1.5 M Tris-HCl, pH 8.8:	25.0ml	10% SDS:	1.0ml	Deionized H ₂ O:	46.2ml
	4X Resolving Buffer:	25.0ml		1.5 M Tris-HCl, pH 8.8:		25.0ml				
	Deionized H ₂ O:	47.2ml		10% SDS:		1.0ml				
10%	AccuGel 29:1 30%:	33.3ml	AccuGel 29:1 30%:	33.3ml	1.5 M Tris-HCl, pH 8.8:	25.0ml	10% SDS:	1.0ml	Deionized H ₂ O:	39.6ml
	4X Resolving Buffer:	25.0ml		1.5 M Tris-HCl, pH 8.8:		25.0ml				
	Deionized H ₂ O:	40.6ml		10% SDS:		1.0ml				
12%	AccuGel 29:1 30%:	40.0ml	AccuGel 29:1 30%:	40.0ml	1.5 M Tris-HCl, pH 8.8:	25.0ml	10% SDS:	1.0ml	Deionized H ₂ O:	32.9ml
	4X Resolving Buffer:	25.0ml		1.5 M Tris-HCl, pH 8.8:		25.0ml				
	Deionized H ₂ O:	33.9ml		10% SDS:		1.0ml				
15%	AccuGel 29:1 30%:	50.0ml	AccuGel 29:1 30%:	50.0ml	1.5 M Tris-HCl, pH 8.8:	25.0ml	10% SDS:	1.0ml	Deionized H ₂ O:	22.9ml
	4X Resolving Buffer:	25.0ml		1.5 M Tris-HCl, pH 8.8:		25.0ml				
	Deionized H ₂ O:	23.9ml		10% SDS:		1.0ml				

Volumes of AccuGel 29:1 40% and ProtoGel Resolving Buffer To Achieve Common Gel Percentages

% Monomer	METHOD 1		OR	METHOD 2						
	Volume of AccuGel and Resolving Buffer to use			Volume of AccuGel and reagents to use						
6%	AccuGel 29:1 40%:	15.0ml	AccuGel 29:1 40%:	15.0ml	1.5 M Tris-HCl, pH 8.8:	25.0ml	10% SDS:	1.0ml	Deionized H ₂ O:	57.9ml
	4X Resolving Buffer:	25.0ml		1.5 M Tris-HCl, pH 8.8:		25.0ml				
	Deionized H ₂ O:	58.9ml		10% SDS:		1.0ml				
8%	AccuGel 29:1 40%:	20.0ml	AccuGel 29:1 40%:	20.0ml	1.5 M Tris-HCl, pH 8.8:	25.0ml	10% SDS:	1.0ml	Deionized H ₂ O:	52.9ml
	4X Resolving Buffer:	25.0ml		1.5 M Tris-HCl, pH 8.8:		25.0ml				
	Deionized H ₂ O:	53.9ml		10% SDS:		1.0ml				
10%	AccuGel 29:1 40%:	25.0ml	AccuGel 29:1 40%:	25.0ml	1.5 M Tris-HCl, pH 8.8:	25.0ml	10% SDS:	1.0ml	Deionized H ₂ O:	47.9ml
	4X Resolving Buffer:	25.0ml		1.5 M Tris-HCl, pH 8.8:		25.0ml				
	Deionized H ₂ O:	48.9ml		10% SDS:		1.0ml				
12%	AccuGel 29:1 40%:	30.0ml	AccuGel 29:1 40%:	30.0ml	1.5 M Tris-HCl, pH 8.8:	25.0ml	10% SDS:	1.0ml	Deionized H ₂ O:	42.9ml
	4X Resolving Buffer:	25.0ml		1.5 M Tris-HCl, pH 8.8:		25.0ml				
	Deionized H ₂ O:	43.9ml		10% SDS:		1.0ml				
15%	AccuGel 29:1 40%:	37.5ml	AccuGel 29:1 40%:	37.5ml	1.5 M Tris-HCl, pH 8.8:	25.0ml	10% SDS:	1.0ml	Deionized H ₂ O:	35.4ml
	4X Resolving Buffer:	25.0ml		1.5 M Tris-HCl, pH 8.8:		25.0ml				
	Deionized H ₂ O:	36.4ml		10% SDS:		1.0ml				

Pour Stacking Gel

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

AccuGel 29:1 30%	1.3ml
4X ProtoGel Stacking Buffer	2.5ml
Deionized Water	6.1 ml

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.

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