AccuGel™ 19:1
AccuGel™ 29:1

Procedures for gel preparation for DNA and RNA

national diagnostics

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

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.

Mix Gel Solution

Calculate how much AccuGel you need to make your gels by using the table 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.

Equations Needed to Determine AccuGel Formulation

$$V_{A30} = \frac{(X)\,(V_t)}{30} \qquad V_{A40} = \frac{(X)\,(V_t)}{40}$$

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

X = % gel desired

V_t = Total volume of gel casting (solution desired (ml))

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	36
	7M	42	42	42	42	42	42	42	42
	8M	48	48	48	48	48	48	48	48
10X TBE (ml)	0.6X	6	6	6	6	6	6	6	6
	1.0X	10	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

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.

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

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 19:1 30%		**AccuGel 19:1 40%**	
EC-849	450 mL 1 L	EC-850	450 mL 1 L
AccuGel 29:1 30%		**AccuGel 29:1 40%**	
EC-851	450 mL 1 L	EC-852	450 mL 1 L

For additional information and order placement:

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AccuGel™ 29:1

Procedures for gel preparation for SDS-PAGE

national diagnostics

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

AccuGel 29:1 allows you to prepare gels of any percentage monomer. Use the charts on the right to determine the volumes of reagents required for your gel composition. If your percentage gel is not included use the formula below to calculate the AccuGel 29:1 30%, ProtoGel Resolving Buffer and other reagents needed.

$$V_P = \frac{(X)\ (V_t)}{30^*}$$

*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:

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

Add buffer and bring to volume to the amount of AccuGel determined above. Add $0.25V_t$ of ProtoGel Resolving Buffer and enough water to bring volume to V_t.

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 0.1ml 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:

AccuGel 29:1 30%	1.3ml
4X 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.

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

% Monomer	METHOD 1 Volume of AccuGel and Resolving Buffer to use		OR	METHOD 2 Volume of AccuGel and reagents to use	
6%	AccuGel 29:1 30%:	20.0ml		AccuGel 29:1 30%:	20.0ml
	4X ProtoGel 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%	AccuGel 29:1 30%:	26.7ml		AccuGel 29:1 30%:	26.7ml
	4X ProtoGel 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%	AccuGel 29:1 30%:	33.3ml		AccuGel 29:1 30%:	33.3ml
	4X ProtoGel 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%	AccuGel 29:1 30%:	40.0ml		AccuGel 29:1 30%:	40.0ml
	4X ProtoGel 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%	AccuGel 29:1 30%:	50.0ml		AccuGel 29:1 30%:	50.0ml
	4X ProtoGel 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

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

% Monomer	METHOD 1 Volume of AccuGel and Resolving Buffer to use		OR	METHOD 2 Volume of AccuGel and reagents to use	
6%	AccuGel 29:1 40%:	15.0ml		AccuGel 29:1 40%:	15.0ml
	4X ProtoGel Resolving Buffer:	25.0ml		1.5 M Tris-HCl, pH 8.8:	25.0ml
	Deionized H_2O:	58.9ml		10% SDS:	1.0ml
				Deionized H_2O:	57.9ml
8%	AccuGel 29:1 40%:	20.0ml		AccuGel 29:1 40%:	20.0ml
	4X ProtoGel 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
10%	AccuGel 29:1 40%:	25.0ml		AccuGel 29:1 40%:	25.0ml
	4X ProtoGel Resolving Buffer:	25.0ml		1.5 M Tris-HCl, pH 8.8:	25.0ml
	Deionized H_2O:	48.9ml		10% SDS:	1.0ml
				Deionized H_2O:	47.9ml
12%	AccuGel 29:1 40%:	30.0ml		AccuGel 29:1 40%:	30.0ml
	4X ProtoGel Resolving Buffer:	25.0ml		1.5 M Tris-HCl, pH 8.8:	25.0ml
	Deionized H_2O:	43.9ml		10% SDS:	1.0ml
				Deionized H_2O:	42.9ml
15%	AccuGel 29:1 40%:	37.5ml		AccuGel 29:1 40%:	37.5ml
	4X ProtoGel Resolving Buffer:	25.0ml		1.5 M Tris-HCl, pH 8.8:	25.0ml
	Deionized H_2O:	36.4ml		10% SDS:	1.0ml
				Deionized H_2O:	35.4ml

AccuGel 29:1 30%		**AccuGel 29:1 40%**	
EC-851	450 mL	EC-852	450 mL
	1 L		1 L

For additional information and order placement:

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