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Procedures for Gel Preparation with AccuGel 19:1 and AccuGel 29:1

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.

Effective Range of Separation of DNAs and Dye Co-Migration in 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

Effective Range of Separation of DNAs and Dye Co-Migration in 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

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	15.9	16.7	20	26.7	33.3	40
40% AccuGel (ml)		10	10.6	11.9	12.5	15	20	25	30
Urea (g) *For denaturing gels*	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

Mix Gel Solution

Calculate how much AccuGel you need to make your gels by using the table above or the formulas at right. 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. *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.* 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 vacuum for 5 minutes while stirring on a magnetic stirrer.

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

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)

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 20 µl 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 $1^1/_2$ - 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.