Gel Preparation

Preparation of gels from Acrylamide 4x solution 40 % and N,N'-Methylene bisacrylamide 2x solution 2 %

The gel composition is usually expressed as T for the total acrylamide plus Bis concentration and C for the percentage (by weight) of the total monomer (T) that is Bis (or other cross-linker).

It has been found that at a fixed ratio of acrylamide to Bis, pore size varies inversely (and approximately linearly) with total monomer concentration (T). It has also been found that pore size (at a constant T) shows a minimum at C of about 5 %. In practice the apparent sieving effect of a gel may also depend upon the concentration of the catalysts used to initiate polymerization and the time allowed for polymerization. Clearly, reproducibility of results depends on reproducibility of procedure.

Note: *During all these manipulations wear impermeable gloves and wash them before (!) drawing them off.*

T	=	Total concentration of gel components in % : % Acrylamide (AA) + % N,N-Methylenbisacrylamide (Bis)
C	=	Concentration of crosslinker (Bis) in the mixture in % : $\frac{\%\ Bis \times 100}{T}$
$\mathbf{V_{AA}}$	**=**	Volume of AA in ml (related to a 40 % solution)
$\mathbf{V_{Bis}}$	**=**	Volume of Bis in ml (related to a 2 % solution)
$\mathbf{V_t}$	**=**	Total volume of gel components
$\mathbf{V_{AA}}$	=	$\frac{T\ (100-C) \times V_t}{40 \times 100}$
$\mathbf{V_{Bis}}$	**=**	$\frac{T \times C \times V_t}{2 \times 100}$

Pore Size Control

The gel matrix restricts the movement of macromolecules to an extent which is dependent upon the relative sizes of the molecules and the matrix pores. The pore size can be varied by changing the acrylamide content of the gel and to some extent the ratio of acrylamide to bis-acrylamide. Given in the table below are ratios of Acrylamide Stock Solution and Bis Stock Solution to prepare gels ranging from 2 to 20 % T and from 1 to 10 % C to match numerous applications.

Shown in the table below are the required volume (in ml) of Acrylamide 40 % Stock Solution (numbers in upper row) and the corresponding volume (in ml) of Bis 2 % Stock Solution (numbers in lower row). Volume is calculated for a total of 100 ml gel solution.

Example for a gel of T = 9 and C = 5 in 50 ml solution:

V_{AA}	=	$\frac{9\ (100 - 5) \times 50}{40 \times 100}$ = 10.7 ml
V_{Bis}	=	$\frac{9 \times 5 \times 50}{2 \times 100}$ = 11.2 ml

That means you pipette 10.7 ml AA (40 %) and 11.2 ml Bis (2 %), degas, add TEMED plus APS and fill up to 50 ml with degassed water or buffer.

Mixing Table for AA (40 %) and Bis (2 %)

The upper numbers refer to 40 % AA (in ml), the lower numbers refer to 2 % Bis (in ml).
The sample is filled up to 100 ml with water or buffer.

% desired concentration of T	% desired concentration of Bis (C)									
	1	2	3	4	5	6	7	8	9	10
2	4.9 1	4.9 2	4.8 3	4.8 4	4.7 5	4.7 6	4.6 7	4.6 8	4.5 9	4.5 10
4	9.8 2	9.8 4	9.6 6	9.6 8	9.4 10	9.4 12	9.4 14	9.2 16	9 18	9 20
6	14.7 3	14.7 6	14.4 9	14.4 12	14.1 15	14.1 18	13.8 21	13.8 24	13.5 27	13.5 30
8	19.6 4	19.6 8	19.2 12	19.2 16	18.8 20	18.8 24	18.4 28	18.4 32	18.0 36	18.0 40
10	24.5 5	24.5 10	24.0 15	24.0 20	23.5 25	23.5 30	23.0 35	23.0 40	22.5 45	22.5 50
12	29.4 6	29.4 12	28.8 18	28.8 24	28.2 30	28.2 36	27.6 42	27.6 48	27.0 54	27.0 60
14	34.3 7	34.3 14	33.6 20	33.6 28	32.9 35	32.9 42	32.2 49	32.2 56	31.5 63	
16	39.2 8	39.2 16	38.4 24	38.4 32	37.6 40	37.6 48	36.8 56	36.8 64		
18	44.1 9	44.1 18	43.2 27	43.2 36	42.3 45	42.3 54				
20	49	49	48	48	47					

Instructions for preparation of gels from solutions of Acrylamide and N,N´-Methylenebisacrylamide, 40 %(w/v) and 30 % (w/v) in water

Cat. No.	Composition: Acrylamide:Bis	% C	% T	Common Application
10679	19:1	5.0	40	DNA Sequencing
10680	29:1	3.3	40	IEF, SDS-PAGE
10681	37.5:1	2.6	40	SDS-PAGE
10687	29:1	3.3	30	IEF, SDS-PAGE
10688	37.5:1	2.6	30	SDS-PAGE

Our SERVA acrylamide-bis solutions are applicable to all PAGE methods. The Acrylamide-bis solution 29:1 is also suitable for preparation of a gel according to Schägger and von Jagow [1)].

Storage:	at 2 - 8 °C
Shelf life time	9 months if bottle stays unopened (ref. to best-before-use date)
Packaging size:	500 ml and 4 x 500 ml
Packaging material:	polyethylene
Safety information:	Acrylamide is a neurotoxin. Wear appropriate clothing (gloves, coat, goggles). Contact with skin may cause irritation. Wash with sufficient amounts of water, if swallowed: drink large amounts of water and vomit. Consult a doctor. For further information see the material safety data sheet.

Reference: 1) Schägger, H. & Jagow, U. (1987) ANAL. BIOCHEM. **166**, 366-379

Standard protocol for casting DNA sequencing gels

Amount of gel solution (70 ml) is sufficient for a sequencing gel 400 mm x 330 mm x 0.4 mm.

Product	40 % Concentrate for final concentration of gel (% T)			30 % Concentrate for final concentration of gel (% T)		
	4 %	**6 %**	**8 %**	**4 %**	**6 %**	**8 %**
Acrylamide-bis solution *	7.0 ml	10.5 ml	14.0 ml	9.4 ml	14 ml	18.7 ml
TBE buffer, 10X (cat.no. 42557)	7.0 ml	7.0 ml	7.0 ml	7.0 ml	7.0 ml	7.0 ml
Urea (cat.no. 24524)	29.4 g	29.4 g	29.4 g	29.4 g	29.4 g	29.4 g
Water demin. or aqua dest.	ad 70 ml	ad 70 ml	ad 70 ml	ad 70 ml	ad 70 ml	ad 70 ml
TEMED (cat.no. 35925, as 10 % solution)	0.29 ml	0.29 ml	0.29 ml	0.29 ml	0.29 ml	0.29 ml
APS (cat.no. 13375, as 10 % solution)	0.29 ml	0.29 ml	0.29 ml	0.29 ml	0.29 ml	0.29 ml

* The composition of the acrylamide-bis solution (proportion acrylamide:crosslinker) depends on the application of choice. For a standard sequencing gel an acrylamide/bis solution 19:1 (cat.no. 10679) is recommended.

Preparation:

- Mix acrylamide-bis solution, buffer, urea and approx. 20 ml of water in a beaker. Make sure the urea is dissolved completely. Adjust the volume to 70 ml with water.
- Eventually deaerate solution briefly (1 to 3 minutes ad vacuo).
- Add 10 % TEMED solution and ammonium persulfate(APS) solution (10 % in water), swirl gently to mix without incorporating air into the mixture.
- Pour gel.

Standard protocol for casting SDS-PAGE gels

(modified according to Laemmli[1])

Amount of gel solutions (10 ml of separating gel and 10 ml of stacking gel) is sufficient to cast 2 vertical slab gels (100 mm x 80 mm x 1.4 mm)

Reference:
1) Laemmli, U.K. (1970) NATURE **227,** 680-685

Product	**For final concentration of gel (% T):**									
	Separating gel (10 ml)								**Stacking gel (10 ml)**	
	7 %		**10 %**		**12,5 %**		**15 %**		**5 %**	
	30 %*	**40 %***	**30 %**	**40 %**	**30 %**	**40 %**	**30 %**	**40 %**	**30 %**	**40 %**
Acrylamide-bis solution %[a)]	2.33 ml	1.75 ml	3.33 ml	2.50 ml	4.17 ml	3.13 ml	5.00 ml	3.75 ml	1.67 ml	1.25 ml
Separating buffer 4 x	2.50 ml	2.50 ml	2.50 ml	2.50 ml	2.50 ml	2.50 ml	2.50 ml	2.50 ml	-	-
Stacking gel buffer, 4 x	-	-	-	-	-	-	-	-	2.50 ml	2.50 ml
Water demin. or aqua dest.	5.07 ml	5.65 ml	4.03 ml	4.90 ml	3.29 ml	4.27 ml	2.46 ml	3.65 ml	5.79 ml	6.15 ml
SDS (cat.no. 20763, as 10 % solution)	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
TEMED (cat.no. 35925)	0.015 ml	0.015 ml	0.015 ml	0.015 ml	0.015 ml	0.015 ml	0.015 ml	0.015 ml	0.015 ml	0.015 ml
APS (cat.no. 13375)	0.03 ml	0.03 ml	0.03 ml	0.03 ml	0.03 ml	0.03 ml	0.03 ml	0.03 ml	0.03 ml	0.03 ml

* The first column contains volumes when working with a 30 % concentrate, the second one those for working with a 40 % concentrate.

[a)] The composition of the acrylamide/bis solution (proportion acrylamide:crosslinker) depends on the application of choice. For a standard SDS PAGE gel an acrylamide/bis solution 29:1 (cat.no. 10680) resp. 37.5:1 (cat.no. 10681) is recommended.

Preparation:

1. Mix acrylamide/bis solution, buffer and water in separate beakers.

2. Deaerate the solutions briefly (1 to 3 minutes in vacuo).

3. Add to separating gel solution: 10 % SDS solution (w/v in water), TEMED and APS solution (w/v 10 % of ammoniumpersulfate), swirl gently to mix without incorporating air into the mixture.

4. Casting the separating gel: fill slabgel sandwich with separating gel solution up to approx. two thirds. The remaining volume for the stacking gel should refer to aseparation distance of 1.5-fold of the depth of the wells of the comb to be used. Overlay with isopropanol immediately (0.1 ml), let polymerize (approx. 15 - 20 min.).

5. Add 10 % SDS in water to the stacking gel solution, thenTEMED and 10 % ammonium persulfate solution (APS),swirl gently to mix without incorporating air into the mixture.

6. Casting the stacking gel: fill up the slab gel sandwich to the top and let polymerize.

Separating gel buffer, 4x (1.5 M Tris/HCl pH 8.8), 100 ml:
Dissolve 18.2 g Tris (cat.no. 37190) in water (bidest.) and adjust pH to 8.8 with approx. 2.8 ml hydrochloric acid 32 %

Stacking gel buffer, 4 x (0.5 M Tris/HCl pH 6.8), 100 ml:
Dissolve 6.1 g Tris (cat.no. 37190) in water (bidest.) and adjust pH to 6.8 with approx. 4.4 ml hydrochloric acid 32 %

Sample buffer 2 x, 10 ml:
Stacking gel buffer 4 x 2.5 ml
SDS, 10 % (cat.no. 20763 as 10 % solution in water) 4.0 ml
Glycerol (cat.no. 23176) 2.3 ml
2-Mercaptoethanol (cat.no. 28625) 0.5 ml
Bromophenolblue (cat.no. 15375) 1.0 mg
Water (bidest.) 0.7 ml

Running buffer 10x, 1000 ml:
Tris (cat.no. 37190) 30 g
Glycine (cat.no. 23390) 144 g
SDS (cat.no. 20760) 10 g
Water (bidest.) ad 1000 ml