Technical Info

Well advised with Roth.

PAGE

Polyacrylamide Gel Electrophoresis

Gel electrophoresis remains to be a technology in modern-day bioanalytics which cannot be replaced by any other. In the vast majority of cases, Vertical gels are produced from acrylamide which with bis-acrylamide and after the addition of ammonium persulphate (radical donator) and TEMED (catalyst) form a very fine and extremely constant network by means of a radical chain reaction.

Application, however, is made more difficult as acrylamide is a very strong nerve poison, the carcinogenic and mutating properties of which have been clearly proven in animal testing. Acrylamide is primarily resorbed through the skin, but above all, it is the absorption of dust in the respiratory tracts or through the facial mucous membrane when weighing out the powder which causes the most serious problems. ROTIPHORESE® ready-to-use solutions provide the perfect remedy. The solutions, which have been stringently controlled with regard to their acrylamide content and pH, are significantly less dangerous in their application.
They are subsequently very easy to use and enable reproducible and high-resolution gel electrophoresis.
In our Roth range you will also find all additional reagents needed, such as TEMED, APS, SDS or gel electrophoresis buffers which form a perfect, coordinated team (see below).

Recommended Applications

Separation of	Recommended gel solution	% C	Acrylamide / bisacrylamide
Nucleic acids	Gel 40 (3030)	5	19:1
	NF-acrylamide/bis-solution 40 % (A516)	5	19:1
	Sequencing gel concentrate 25 % (3043)	5	19:1
Nucleic acids and proteins	Gel 40 (A515)	3.3	29:1
	NF-acrylamide/bis-solution 40 % (A121)	3.3	29:1
	NF-acrylamide/bis-solution 30 % (A124)	3.3	29:1
Proteins	Gel 30 (3029)	2.6	37.5:1
	Gel 40 (T802)	2.6	37.5:1

Gel Preparation

Polyacrylamide gel electrophoresis (PAGE) is used for both high-resolution nucleic acid gels (e.g. sequencing gels) as well as for almost all protein gels. Nucleic acid is, as a rule, separated in a TBE-buffer system, whereas proteins are mixed with SDS for a uniform negative load and separated with Tris/Glycine buffer (SDS-PAGE). A detailed description may be found in e.g. Sambrook and Russel's *Molecular Cloning 3rd Edition*, CSHL Press New York, 2004 or in *Proteins: Standard Methods of Molecular and Cell Biology*, Eckert and Kartenbeck, Springer Verlag Heidelberg, 1997.

The following tables provide a reference for typical gel mixes in SDS-gel electrophoresis (A) and the separation of nucleic acids (B).

A) SDS-PAGE

Separation range of SDS-gels

Acrylamide concentration (%)	6 %	8 %	10 %	12 %	15 %
Separation range (kD)	50-200	30-95	20-80	12-60	10-43

Resolving gel (data apply to 20 ml gel solution)

30 % acrylamide mix	**Gel concentration**	6 %	8 %	10 %	12 %	15 %
	Aqua dest. (ml)	10.6	9.3	7.9	6.6	4.6
	30 % acrylamide mix (ml)	4	5.3	6.7	8	10
	Tris (1.5 M, pH 8.8) (ml)	5	5	5	5	5
40 % acrylamide mix	**Gel concentration**	6 %	8 %	10 %	12 %	15 %
	Aqua dest. (ml)	11.6	10.6	9.6	8.6	7.1
	40 % acrylamide mix (ml)	3	4	5	6	7.5
	Tris (1.5 M, pH 8.8) (ml)	5	5	5	5	5

Add in this order:

200 µl 10 % SDS solution (mix carefully, avoid bubbles)

200 µl 10 % ammonia persulphate solution (prepare freshly)

20 µl TEMED (mix carefully, avoid bubbles)

Pour gel immediately and overlay with isopropanol

Stacking gel (data apply to 5 % gels)

30 % acrylamide mix	**Gel volume**	1 ml	3 ml	5 ml	8 ml	10 ml
	Aqua dest. (ml)	0.68	2.1	3.4	5.5	6.8
	30 % acrylamide mix (ml)	0.17	0.5	0.83	1.3	1.7
	Tris (1.0 M, pH 6.8) (ml)	0.13	0.38	0.63	1	1.25
	SDS (10 % solution) (µl)	10	30	50	80	100
	APS (10 % solution*) (µl)	10	30	50	80	100
	TEMED (µl)	1	3	5	8	10
40 % acrylamide mix	**Gel volume**	1 ml	3 ml	5 ml	8 ml	10 ml
	Aqua dest. (ml)	0.725	2.185	3.645	5.84	6.3
	40 % acrylamide mix (ml)	0.125	0.375	0.625	1	1.25
	Tris (1.0 M, pH 6.8) (ml)	0.13	0.38	0.63	1	1.25
	SDS (10 % solution) (µl)	10	30	50	80	100
	APS (10 % solution*) (µl)	10	30	50	80	100
	TEMED (µl)	1	3	5	8	10

* prepare freshly!

Be careful to mix the solution thoroughly before and after addition of SDS and TEMED.
Avoid bubbles. Pour the stacking gel immediately and insert the comb carefully.

B) Separation of nucleic acids

Denaturing TBE gels for separation of single stranded nucleic acids (e.g. sequencing gels)
(data apply to 100 ml gel solution)

25 % sequencing gel concentrate with urea	**Gel concentration**	4 %	6 %	8 %
	Sequencing gel diluent (ml)*	74	66	58
	25 % sequencing gel concentrate (ml)	16	24	32
30 % acrylamide mix (29:1)	**Gel concentration**	4 %	6 %	8 %
	Aqua dest. (ad 90 ml) (ml)*	app. 52	app. 45	app. 39
	30 % acrylamide mix (ml)	13.3	20	26.5
	Urea (g)**	42	42	42
40 % acrylamide mix (19:1 or 29:1)	**Gel concentration**	4 %	6 %	8 %
	Aqua dest. (ad 90 ml) (ml)*	app. 55	app. 50	app. 45
	40 % acrylamide mix (ml)	10	15	20
	Urea (g)**	42	42	42

*If required for resolution of secondary structures the gel may be supplemented with formaldehyde by replacing 25 ml aqua dest. with 25 ml formaldehyde.

**Results in gels with 42 % urea (7 M)

Add in this order:

10 ml 10 x TBE buffer*** (mix carefully, avoid bubbles, degas if required)

400 µl 10 % ammonia persulphate solution (prepare freshly)

50 µl TEMED (mix carefully, avoid bubbles)

Pour gel immediately and insert the comb carefully

***Results in 45 % urea if sequencing gel concentrate and sequencing gel diluent are used. If 50% urea are required replace 10 x TBE by the ready-to-use sequencing gel buffer concentrate with 50 % urea (Ord. No. 3050.1).

TBE gels for electrophoresis of ds nucleic acid (data apply to 100 ml gel solution)

30 % acrylamide mix (29:1)	**Gel concentration**	6 %	10 %	15 %
	Aqua dest. (ml)	69	56	39
	30 % acrylamide mix (ml)	20	33	50
40 % acrylamide mix (19:1 or 29:1)	**Gel concentration**	6 %	10 %	15 %
	Aqua dest. (ml)	74	64	51.5
	40 % acrylamide mix (ml)	15	25	37.5

Add in this order:

10 ml 10 x TBE buffer (mix carefully, avoid bubbles, degas if required)

1 ml 10 % ammonia persulphate solution (prepare freshly)

60 µl TEMED (mix carefully, avoid bubbles)

Pour gel immediately and insert the comb carefully

Variable Regulation of Pore Sizes Using ROTIPHORESE® Gel A and B

The pore size of acrylic amide gels can be varied by regulating the total gel concentration (% T) and the percentage of the crosslink (% C). You need the acrylamide stock solutions ROTIPHORESE® Gel A and Gel A-40, respectively, and the bisacrylamide stock solution ROTIPHORESE® Gel B. With these solutions the T/C ratio can be produced as required:

V_t	=	Total volume of gel casting solution (ml)	
T	=	Gel concentration in %	= % Acrylamide + % Bisacrylamide
C	=	% Crosslinking	= (% Bisacrylamide x 100) / T
V_a	**=**	**Volume Gel A/A-40 in ml**	**V_b = Volume Gel B in ml**

Applying for Gel A (30 % solution):

$V_a = (T \times (100 - C) \times V_t) / 3000$ $V_b = (T \times C \times V_t) / 200$ **for T ≤ 20**

Applying for Gel A-40 (40 % solution):

$V_a = (T \times (100 - C) \times V_t) / 4000$ $V_b = (T \times C \times V_t) / 200$ **for T ≤ 25**

Example Gel A: To prepare 100 ml gel solution with 10 % T and 2.7 % C, calculate as follows:

$V_a = (10 \times (100 - 2.7) \times 100) / 3000$ = **32.433 ml Gel A**

$V_b = (10 \times 2.7 \times 100) / 200$ = **13.500 ml Gel B**

Combine 32.43 ml Gel A and 13.5 ml Gel B and fill up the volume to 100 ml with the usually used buffer. Degas and add APS and TEMED, mix thoroughly while avoiding bubbles and pour the gel.

Example Gel A-40: To prepare 100 ml gel solution with 10 % T and 2.7 % C, calculate as follows:

$V_a = (10 \times (100 - 2.7) \times 100) / 4000$ = **24.325 ml Gel A-40**

$V_b = (10 \times 2.7 \times 100) / 200$ = **13.500 ml Gel B**

Combine 24.325 ml Gel A and 13.5 ml Gel B and fill up the volume to 100 ml with the usually used buffer. Degas and add APS and TEMED, mix thoroughly while avoiding bubbles and pour the gel.

ROTIPHORESE® Gel Solutions

Ready-to-use acrylamide/bisacrylamide mixtures

ROTIPHORESE® Gel 30 (37.5:1): 30 % acrylamide/bisacrylamide, mixing ratio 37.5:1.
Art. No. 3029.2 (250 ml), 3029.3 (500 ml), 3029.1 (1 l)

ROTIPHORESE® Gel 40 (19:1): 40 % acrylamide/bisacrylamide, mixing ratio 19:1.
Art. No. 3030.2 (250 ml), 3030.1 (1 l)

ROTIPHORESE® Gel 40 (29:1): 40 % acrylamide/bisacrylamide, mixing ratio 29:1.
Art. No. A515.2 (250 ml), A515.1 (1 l)

ROTIPHORESE® Gel 40 (37.5:1): 40 % acrylamide/bisacrylamide, mixing ratio 37.5:1.
Art. No. T802.2 (250 ml, T802.1 (1 l)

Acrylamide- and bisacrylamide solution, ready-to-mix

ROTIPHORESE® Gel A: 30 % acrylamide solution. Art. No. 3037.2 (250 ml), 3037.1 (1 l)

ROTIPHORESE® Gel A-40: 40 % acrylamide solution. Art. No. 7748.1 (250 ml), 7748.2 (1 l)

ROTIPHORESE® Gel B: 2 % bisacrylamide solution. Art. No. 3039.2 (250 ml), 3039.1 1 (1 l)

Acrylamide/bisacrylamide mixtures for automated sequencing (fluorescence free)

ROTIPHORESE® NF-acrylamide/bis- solution 40 % (19:1):
ready-to-use 40 % acrylamide/bisacrylamide, mixing ratio 19:1. Art. No. A516.1 (250 ml)

ROTIPHORESE® NF-acrylamide/bis- solution 40 % (29:1):
ready-to-use 40 % acrylamide/bisacrylamide, mixing ratio 29:1. Art. No. A121.1 (250 ml)

ROTIPHORESE® NF-acrylamide/ bis- solution 30 % (29:1):
ready-to-use 30 % acrylamide/bisacrylamide, mixing ratio 29:1. Art. No. A124.1 (250 ml), A124.2 (1 l)

Ready-to-use sequencing gel solutions

ROTIPHORESE® DNA sequencing system: (1 l sequencing gel concentrate, 1 l sequencing gel diluent, 250 ml sequencing gel buffer) Art. No. A431.1 (1 Kit)

ROTIPHORESE® sequencing gel concentrate:
25 % acrylamide/bisacrylamide, mixing ratio 19:1 and 50 % urea. Art. No. 3043.2 (100 ml), 3043.1 (1 l)

ROTIPHORESE® sequencing gel diluent:
50 % urea in water for dilution of the sequencing gel concentrate. Art. No. 3047.1 (1 l)

ROTIPHORESE® sequencing gel buffer concentrate: 50 % urea in 10x TBE. Art. No. 3050.1 (250 ml)

Additional Reagents and Solutions

Product	Art. No.
TEMED	2367
APS	9592
Acrylamide p.a., 4 x crist.	7906
Acrylamid, BioScience, for genetic engineering	0189
Acrylamid, 2x crist.	7871
Bisacrylamide	7867
Tris p.a.	4855
Glycine p.a.	3908
SDS ultra pure	2326
SDS Pellets, ROTIPHORESE®-Grade, for electrophoresis	8029
ROTI®Stock 20 % SDS Fertiglösung	1057
Urea	X999
ROTIPHORESE® NF Urea, fluorescence free	A120
ROTIPHORESE® NF 10 x TBE Buffer, fluorescence free	A118
ROTIPHORESE® 10 x TBE Puffer	3061
ROTIPHORESE® 10 x SDS PAGE Buffer	3060
ROTI®Load 1, reducing, protein gel load	K929
ROTI®Load 2, non-reducing, protein gel load	K930
ROTI®Load 3, non-reducing, protein gel load with LDS	3359
ROTI®Load DNA (with glycerol), 6x conc.	X904
ROTI®Load DNA (with ficoll), 6x conc.	X905
ROTI®Load DNAstain 1, simultaneous gel staining, for large fragments	5783
ROTI®Load DNAstain 2, simultaneous gel staining, for middle sized fragments	5784
ROTI®Load DNAstain 3, simultaneous gel staining, for small fragments	6472

For further data, safety information, or package sizes please see our catalogue or online at www.carlroth.com

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