Rotiphorese® Sequencing Gel Systems and Concentrates

A431, 3043, 3047, 3050

Since the end of the 1970s, DNA-sequencing has turned out to be one of the most important methods applied within Life Sciences, and in 1995 it marked the beginning of the genomics era. The most common sequencing methods are those based on Allan Maxam's and Walter Gilbert's[1] base specific chemical cleavage, which is, however, extremely complex and was therefore quickly replaced by the less complicated chain termination method according to Frederick Sanger and Alan Coulson[2]. Sanger was awarded the Nobel Prize in Chemistry for his work on DNA-sequencing together with Gilbert in 1980. Although marked mainly by radioisotopes a decade ago, the vast majority of sequences are now determined via fluorescence signals. Since 2000 the newer method of pyrosequencing is also being used more and more frequently (generation of a flash via luciferase reaction by integration of a complementary nucleotide). This method enables, for example, even highly compressing sequences to be read without difficulty.

[1]Maxam, A. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. U.S.A.* 74:560-4.
[2]Sanger F., Nicklen S. und Coulson A.R. (1977) *Proc. Natl. Acad. Sci. U.S.A.* 74:5463-7.
[3]Nyrén P. (1987) *Anal Biochem.* 167:235-8.

1.) Reagents

Ready-to-use sequencing gel system

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. 3043.1 (1 l), 3043.2 (100 ml)

Rotiphorese® sequencing gel diluent: 50 % urea. Art. No 3047.1 (1 l)

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

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)

Rotiphorese® NF urea, fluoreszenzfrei. Art. No. A120.1 (1 kg)

Rotiphorese® NF 10xTBE buffer. Art. No. A118.1 (2.5 l)

Reagents for manual sequencing

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

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

Urea. Ord. No. X999.2 (1 kg)

10xTBE buffer stock solution. Ord. No. 3061.1 (1 l)

Acrylamide- and bisacrylamide solution, ready-to-mix

Rotiphorese® Gel A: 30 % acrylamide solution. Art. No. 3037.1 (1 l)
Rotiphorese® Gel B: 2 % bisacrylamide solution. Art. No. 3039.1 (1 l), 3039.2 (250 ml)
Reagents for Polymerisation
TEMED. Art. No. 2367.1 (100 ml)
APS. Art. No. 9592.1 (100 g)
For further informations or other packages please see our catalogue or the Internet at www.carlroth.com

2.) Application:
(All data meant for 100 ml gel mix)

A) Using the Rotiphorese®-sequencing gel system
Sequencing gel concentrate: 25 % acrylamide / bisacrylamide in 50 % urea
Sequencing gel diluent: 50 % urea
Sequencing buffer concentrate: 10x TBA in 50 % urea

For gels with 50 % (0.83 M) urea:

	Gel concentration	**4 %**	**6 %**	**8 %**
Sequencing gel system	Sequencing gel diluent (ml)	74	66	58
	Sequencing gel concentrate (ml)	16	24	32
	Sequencing buffer concentrate (ml)	10	10	10

For gels with 45 % (7.5 M) urea:

	Gel concentration	**4 %**	**6 %**	**8 %**
Sequencing gel system	Sequencing gel diluent (ml)	74	66	58
	Sequencing gel concentrate (ml)	16	24	32
	10x TBE buffer stock sol. (ml)	10	10	10

or

	Gel concentration	**4 %**	**6 %**	**8 %**
Sequencing gel system	Sequencing gel diluent (ml)	64	56	48
	Sequencing gel concentrate (ml)	16	24	32
	Sequencing buffer concentrate (ml)	10	10	10
	Aqua dist. (ml)	10	10	10

For gels with 40 % (6.7 M) urea:

	Gel concentration	**4 %**	**6 %**	**8 %**
Sequencing gel system	Sequencing gel diluent (ml)	54	46	38
	Sequencing gel concentrate (ml)	16	24	32
	Sequencing buffer concentrate (ml)	10	10	10
	Aqua dist. (ml)	20	20	20

Polymerisation:
Add in this order:
500 µl 10 % ammonia persulphate solution (prepare freshly)
50 µl TEMED
Mix carefully, avoiding bubbles. Pour gel immediately, insert comb carefully.

B) Using Rotiphorese® ready-made gel solutions

Rotiphorese® gel solution 40: 40 % mixture of acrylamide / bisacrylamide 19:1 or 29:1 **without urea**
Sequencing gel diluent: 50 % Urea
Sequenziergel-Pufferkonzentrat: 10x TBA in 50 % Urea

For gels with 50 % (8.3 M) Urea:

40 % acrylamide mix (Rotiphorese® gel solution 19:1 or 29:1)	**Gel concentration**	**4 %**	**6 %**	**8 %**
	Aqua dist. (ml)	0	x	x
	Rotiphorese® gel solution 40 (ml)	10	x	x
	Sequencing gel diluent (ml)	80	x	x
	Sequencing buffer concentrate (ml)	10	x	x

or

40 % acrylamide mix (Rotiphorese® gel solution 19:1 or 29:1)	**Gel concentration**	**4 %**	**6 %**	**8 %**
	Aqua dist. (ad 100 ml) (ml)	ca. 50	ca. 45	ca. 40
	Rotiphorese® gel solution 40 (ml)	10	15	20
	Urea (g)	50	50	50
	10x TBE buffer stock sol. (ml)	10	10	10

In case secondary structures of DNA stands shall be reduced, formamide may be added: 25 ml per 100 ml gel mixture. Reduce water amount correspondingly.

For gels with 45 % (7.5 M) Urea:

40 % acrylamide mix (Rotiphorese® gel solution 19:1 or 29:1)	**Gel concentration**	**4 %**	**6 %**	**8 %**
	Aqua dist. (ml)	5	0	x
	Rotiphorese® gel solution 40 (ml)	10	15	x
	Sequencing gel diluent (ml)	75	75	x
	Sequencing buffer concentrate (ml)	10	10	x

or

40 % acrylamide mix (Rotiphorese® gel solution 19:1 or 29:1)	**Gel concentration**	**4 %**	**6 %**	**8 %**
	Aqua dist. (ad 100 ml) (ml)	ca. 54	ca. 49	ca. 44
	Rotiphorese® gel solution 40 (ml)	10	15	20
	Urea (g)	45	45	45
	10x TBE buffer stock sol. (ml)	10	10	10

In case secondary structures of DNA stands shall be reduced, formamide may be added: 25 ml per 100 ml gel mixture. Reduce water amount correspondingly.

For gels with 40 % (6.7 M) Urea:

40 % acrylamide mix (Rotiphorese® gel solution 19:1 or 29:1)	**Gel concentration**	**4 %**	**6 %**	**8 %**
	Aqua dist. (ml)	10	5	0
	Rotiphorese® gel solution 40 (ml)	10	15	20
	Sequencing gel diluent (ml)	70	70	70
	Sequencing buffer concentrate (ml)	10	10	10

or

40 % acrylamide mix (Rotiphorese® gel solution 19:1 or 29:1)	**Gel concentration**	**4 %**	**6 %**	**8 %**
	Aqua dist. (ad 100 ml) (ml)	ca. 58	ca. 53	ca. 48
	Rotiphorese® gel solution 40 (ml)	10	15	20
	Urea (g)	40	40	40
	10x TBE buffer stock sol. (ml)	10	10	10

In case secondary structures of DNA stands shall be reduced, formamide may be added: 25 ml per 100 ml gel mixture. Reduce water amount correspondingly.

In case a 30 % gel solution shall be used, urea must be added in powdered form. The amount of Rotiphorese® gel solution must be increased by 1/3 (to 13.3, 20, or 26.5 %, respectively). Reduce water amount correspondingly.

Polymerisation:
Add in this order:
500 µl 10 % ammonia persulphate solution (prepare freshly)
50 µl TEMED
Mix carefully, avoiding bubbles.
Pour gel immediately, insert comb carefully.

3.) 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). Gels with every desired T/C ratio can be produced with Rotiphorese® Gel A and B:

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 in ml V_b = Volume Gel B in ml
Applying:
$V_a = (T \times (100-C) \times V_t) / 3000$ $V_b = (T \times C \times V_t) / 200$

Example: In order to prepare 100 ml gel solution with 10 % T and 2.7 % C, calculate as follows:
$V_a = (10 \times (100-2.7) \times 100) / 3000 = 32.43$ ml Gel A
$V_b = (10 \times 2.7 \times 100) / 200 = 13.5$ 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.

4.) Hazard and Precautionary Statements

(Please note safety data given on label and MSDS.)

Sequencing Gels and Concentrates:

Danger H302-H315-H319-H317-H340-H350-H360FD-H372

ROTIPHORESE®DNA sequencing system	**glass**	**1 Kit (2x 1L, 1x 250ml)**	**A431**
ROTIPHORESE®Sequencing gel concentrate	**glass**	**1 L**	**3043.1**
	glass	**100 ml**	**3043.2**
ROTIPHORESE®Sequencing gel diluent	**glass**	**1 L**	**3047.1**
ROTIPHORESE®Sequencing gel buffer concentrate	**glass**	**250 ml**	**3050.1**

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