

Acrylamide/Bisacrylamide (37.5:1), solution

Cat. No. 1 685 821

500 ml

Version 3, September 1999

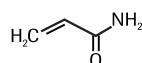
Store at 15-25°C

1. Acrylamide, ultrapure

Product description

Appearance White, crystalline powder

Structure



Formula C₃H₅NO

Molecular weight M_r = 71.08

Purity > 99.9% (from C)

Stability Stable at 15-25°C, protected from light and stored dry.

Safety precautions

Acrylamide is a neurotoxin that is absorbed through the skin. It is classed as a hazardous substance and may cause cancer and genetic damage (Carc. Cat. 2, Mut. Cat. 2). Acrylamide dust is particularly dangerous. A sealed fume cupboard should be used when working with solid acrylamide, and a mask, gloves and eye protection should be worn.

Notes

R 45: May cause cancer
R 46: May cause heritable genetic damage
R 24/25: Toxic in contact with skin and if swallowed
R 48/23/24/25: Toxic: danger of serious damage to health by prolonged exposure through inhalation, contact with skin and if swallowed.
S 53: Avoid exposure – obtain special instructions before use
S 27: Take off immediately all contaminated clothing.
S 45: In case of accident or if you feel unwell, seek medical advice immediately (show the label if possible)

Disposal

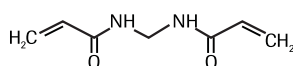
Acrylamide residues or solutions should be polymerized before disposal.

2. Bisacrylamide

Product description

Appearance White, crystalline powder

Structure



Formula C₇H₁₀N₂O₂

Molecular weight M_r = 154.17

Stability Stable at 15-25°C, stored dry and protected from light

3. Acrylamide/bisacrylamide (37.5:1), solution

contains 30% acrylamide (w/v) and 0.8% bisacrylamide (w/v)

Product description

Appearance Clear, colorless solution

Purity Free acrylic acid <0.005%

Stability Stable at 2-8°C, stored protected from light.

Safety precautions As for acrylamide and bisacrylamide

Application

Polyacrylamide gels are commonly used in electrophoresis. This polymer has the advantage of a reproducible pore size and mechanical stability. Depending on the degree of cross-linking, proteins of 6000 to 400 000 D and oligonucleotides of 20 to 100 nucleotides can be separated.

The gels are produced by polymerizing monomeric acrylamide with a crosslinker, usually N,N'-methylene-bis-acrylamide (bisacrylamide) in the presence of a catalyst.

The reproducibility of the pore size depends on this polymerization reaction. The reagents used must therefore be of an appropriate quality.

Working instructions

I. for separation of proteins

Pouring of a gel (15 ml) with the following monomer concentrations (%T), and preparation of a stacking gel (5 ml) according to Laemmli (1).

Required solutions and buffers

Acrylamide solution

Dissolve 30 g acrylamide, ultrapure and 0.8 g bisacrylamide in 100 ml of redist. water. Protected from light, the stock solution is stable for several weeks at 2-8°C.
[Alternatively, the ready-to-use solution can be used (Cat. No. 1 685 821)].

Separating gel buffer (4 ×)

1.5 M Tris-HCl 0.4% SDS, pH 8.8

Stacking gel buffer (1 ×)

0.139 M Tris-HCl, 0.11% SDS, pH 6.8

TEMED

N,N,N',N'-Tetramethylethylenediamine; use undiluted.

10 % Ammonium peroxodisulfate (ammonium persulfate) (APS):

Dissolve 100 mg/ml in redist. water (prepare immediately before use)

Reagent	7.5 % T	10 % T	12.5 % T	15 % T	20 % T	2% T (stacking gel)
Acrylamide solution	3.75 ml	5.0 ml	6.25 ml	7.5 ml	10 ml	0.75 ml
Water, redist.	7.5 ml	6.25 ml	5.0 ml	3.75 ml	1.25 ml	–
Separating gel buffer (4 ×)	3.75 ml	3.75 ml	3.75 ml	3.75 ml	3.75 ml	–
Stacking gel buffer (1 ×)	–	–	–	–	–	4.25 ml
10% Ammonium persulfate	75 µl	75 µl	75 µl	75 µl	75 µl	25 µl
TEMED	75 µl	75 µl	75 µl	75 µl	75 µl	2.5 µl

II. for separation of oligonucleotides (e.g. sequencing)

according to Sambrook, J. et al. (2)

Required solutions and buffers

Acrylamide solution

Dissolve 95 g acrylamide and 5 g bisacrylamide in 125 ml of redist. water with warming to 37°C and make up to 250 ml. Protected from light, the stock solution is stable for several weeks at 2-8°C.

TBE buffer (5 ×)

Dissolve 54 g Tris and 27.5 g boric acid in 500 ml of redist. water. Add 20 ml 0.5 M EDTA, pH 8.0. Make up to 1 l with redist. water. The pH is about 8.3.

10% Ammonium persulfate

Dissolve 100 mg/ml in redist. water (prepare immediately before use).

The following monomer concentrations (%T) are recommended:

Length of the oligonucleotide	% (T)
< 25 nucleotides	19%
25 to 40 nucleotides	15%
40 to 100 nucleotides	12%

Reagent	12% (T)	15% (T)	19% (T)
Urea, EP-MB grade	31.5 g	31.5 g	31.5 g
Acrylamide solution	23.5 ml	29.5 ml	37.5 ml
TBE (5 ×)	15 ml	15 ml	15 ml
Water, redist.	14 ml	8 ml	0 ml

Mix the reagent and warm to 37°C until the urea has dissolved. Add APS and TEMED.

10% Ammonium persulfate	0.45 ml	0.45 ml	0.45 ml
TEMED	35 µl	35 µl	35 µl

Related products, available from Roche Molecular Biochemicals

Application	Product	Cat. No.	Pack size
Denaturing agents			
	Guanidine-hydrochloride crystalline,	1 492 942	500 g
	Guanidine-hydrochloride, ultrapure, solution (8 M)	1 492 926 1 492 934	100 ml 500 ml
	Guanidine thiocyanate	1 685 929	500 g
	Urea, EP-MB grade	1 685 899 1 685 902	1 kg 5 kg
	Sodium dodecyl sulfate (SDS)	1 028 685 1 028 693	100 g 500 g
Protein molecular weight markers			
39.2-200 kD	Protein molecular weight markers premixed, high range	1 495 992	200 µl
14.4-97.4 kD	Protein molecular weight markers premixed, low range	1 495 984	200 µl
14.3-340 kD	Calibration proteins, gel electrophoresis combithek ¹⁾ , high range	1 317 474	8 × 200 µl
3.4-20.1 kD	Calibration proteins, gel electrophoresis combithek, low range	236 292	4 × 200 µl
39.2-212 kD	Calibration proteins, Biotin-labeled premixed, high range	1 624 237	100 µl
12.5-97 kD	Calibration proteins, Biotin-labeled premixed, low range	1 624 229	100 µl

This combination comprises only the most important products related to the product described. Please refer to our latest catalogue for our current product range or contact your local RMB representative directly.

References

- 1 Laemmli, U. K. (1970) *Nature* **227**, 680.
- 2 Sambrook, J.; Fritsch, E. F. & Maniatis T.; *Molecular Cloning* (1989) 2nd edition, Vol. 2, p. 11.23-11.28.

* available from Roche Molecular Biochemicals

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