



Fig. 1. PlusOne™ electrophoresis chemicals.

In polyacrylamide gel electrophoresis (PAGE) and isoelectric focusing (IEF), the quality of the chemicals used is of vital importance to the results obtained. To ensure the highest possible quality and reproducibility, Amersham Biosciences has augmented its selection of electrophoresis consumables with new PlusOne™ chemicals. The PlusOne range includes quality chemicals for gel casting, buffer preparation, gel additives, detergents, stains and glass plate treatment.

Features

- A complete range of chemicals for electrophoresis
- Optimized quality for each electrophoresis technique
- Strict specification control
- Batch testing to ensure reproducibility
- Free from impurities that interfere with polymerization reactions
- Instructions and recipes included

18-1103-56



Fig. 2. Gel casting cassette, gradient mixer and Power supply.

Gel Casting Chemicals

Acrylamide

In PAGE and IEF, the quality of acrylamide is one of the most important factors in obtaining reproducible results.

In PAGE, the acrylamide must be free of impurities such as heavy metals and antioxidants. These will interfere with polymerization and badly affect the gel structure and consequently the mobility of proteins and nucleic acids.

For IEF the charge of the impurities is an important additional factor. Even low concentrations of acrylic acid (> 100 ppm) will increase electroendosmosis and reduce the stability of pH gradients.

PlusOne acrylamide is available in three qualities, optimized for PAGE, DNA/PAGE and IEF respectively.

PlusOne Acrylamide PAGE contains extremely low concentrations of impurities that interfere with the polymerization reaction. It is specially suitable for SDS and native PAGE techniques.

PlusOne Acrylamide DNA/PAGE is specially developed for the separation of restriction fragments and oligonucleotides derived from sequencing reactions.

PlusOne Acrylamide IEF is outstanding for use in IEF and DNA sequencing work. In addition to the absence of polymerization interfering impurities, Acrylamide IEF is also essentially free of charged acrylamide derivatives (< 20 ppm) and gives very stable pH gradients.

Analysis results

Acrylamide	IEF	DNA/PAGE	PAGE
M.W.	71.08	71.08	71.08
Minimum assay	>99.9%	>99.9%	
Acrylic acid	<0.002%	<0.002%	<0.05%
pH in 5% Aq solution	5.0–6.5	5.0–6.5	>5.0
Conductivity	<2.0	<2.0	
	$\mu\text{mho cm}^{-1}$	$\mu\text{mho cm}^{-1}$	
E 290 nm 1% Aq	<0.15	<0.15	<0.5
Water insoluble material	<0.005%	<0.005%	

PlusOne acrylamide is available as a powder, pre-mixed powder with bisacrylamide (ReadyMix) and as solutions and pre-mixed solutions (ReadySol).

Powder

Dry powder is the most stable form of acrylamide, with a shelf life of more than three years.

ReadyMix

ReadyMix is a pre-weighed mixture of acrylamide and bisacrylamide in powder form. ReadyMix eliminates errors in weighing and the risk of inhaling acrylamide and bisacrylamide.

Solution

Solutions of acrylamide are convenient and considerably reduce the risk of acrylamide dust in the air.

ReadySol

ReadySol, a solution of acrylamide and bisacrylamide, is convenient and fast with minimal preparation and handling. ReadySol ensures a guaranteed composition of the solution.

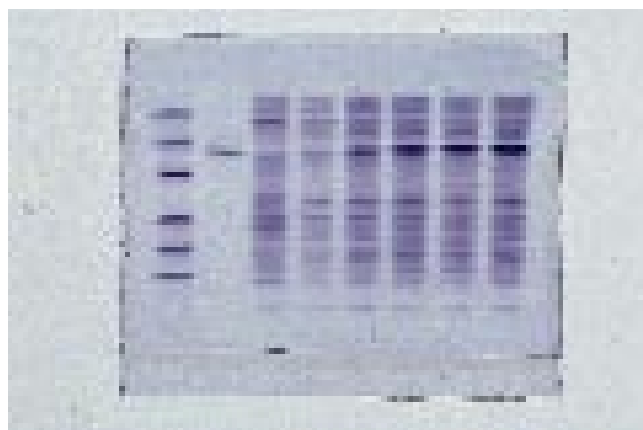


Fig. 3. SDS electrophoresis of *E. Coli* extract and Low Molecular Weight marker kit.

Ordering information

Product	Use	Quantity	Code No.
Acrylamide IEF	IEF, PAGE, Sequencing	250 g	17-1300-01
Acrylamide IEF	IEF, PAGE, Sequencing	1 000 g	17-1300-02
Acrylamide IEF 40% solution	IEF, PAGE	1 000 ml	17-1301-01
ReadyMix IEF 40.25 g acrylamide 1.25g bisacrylamide	IEF	41.5 g Add 100 ml water	17-1309-01
ReadySol IEF T40 C3	IEF	1 000 ml	17-1310-01
Acrylamide PAGE	PAGE	250 g	17-1302-01
Acrylamide PAGE	PAGE	1 000 g	17-1302-02
Acrylamide PAGE 40% Solution	PAGE	1 000 ml	17-1303-01
ReadyMix DNA/PAGE 196.6 g acrylamide 10.4g bisacrylamide	PAGE, Sequencing	207 g Add 500 ml water	17-1307-01
ReadySol DNA/PAGE T40 C5	PAGE, Sequencing	1 000 ml	17-1308-01

ReadyMix should be stored dry and dark at room temperature. Shelf life is 3 years. Acrylamide solutions should be stored dark in the refrigerator at 4 °C–8 °C Shelf life is 24 months. ReadySol should be stored dark in the refrigerator at 4 °C–8 °C. Shelf life is 12 months.

N,N'-methylenebisacrylamide

The special PlusOne bisacrylamide is quality matched to PlusOne acrylamide. Together they form high quality polyacrylamide gels.

Analysis results

N,N'-methylenebisacrylamide	
pH in 2.5% Aq. solution	>5.0
E 290 nm 1% Aq. solution	<0.2
Acrylic Acid (HPLC)	<0.02%

PlusOne bisacrylamide is available as dry powder and as pre-made solutions. Dry powder is the most stable and economical form of bisacrylamide. It gives the flexibility of preparing any concentration solution and ratio to acrylamide. Solutions of bisacrylamide are the most convenient and safe way of handling this chemical. In addition, solutions save time and reduce errors.

Ordering information

Product	Use	Quantity	Code No.
N,N'-Methylene-bisacrylamide	IEF, PAGE, Sequencing	25 g	17-1304-01
N,N'-Methylene-bisacrylamide	IEF, PAGE, Sequencing	100 g	17-1304-02
N,N'-Methylene-bisacrylamide 2% solution	IEF, PAGE, Sequencing	1 000 ml	17-1306-01

Powders of bisacrylamide should be stored dry and dark at room temperature. Shelf life is 3 years. Solutions of bisacrylamide are best stored dark at 4 °C–8 °C. Shelf life is 24 months.

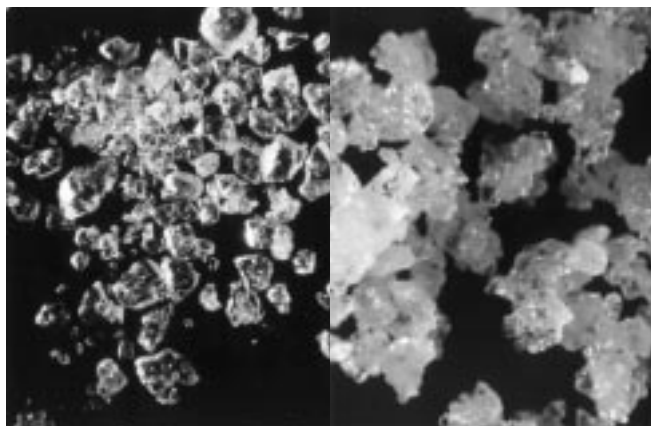


Fig. 4. Ammonium persulphate crystals. Left, fresh and dry. Right, after hygroscopic uptake of water.

Ammonium persulphate

Ammonium persulphate is the initiator of choice for the polymerization of acrylamide. The high quality of PlusOne ammonium persulphate allows start and completion of the polymerization reaction at concentrations as low as 1.0–2.0 mM.

Analysis results

Ammonium persulphate	
M.W.	228.20
Minimum assay	>99%
Copper	<0.002%
Iron	<0.001%
Heavy metals(Pb)	<0.002%
Manganese	<0.00005%

Ordering information

Product	Use	Quantity	Code No.
Ammonium persulphate	IEF, PAGE, Sequencing	25 g	17-1311-01

Ammonium persulphate should be stored dark and dry at room temperature.

TEMED

TEMED (N,N,N',N'-tetramethyl-ethylenediamine) is a catalyst in the polymerization of acrylamide. It is a hygroscopic colourless liquid which is easily oxidised to yellow products. Yellow products have a reduced catalytic activity and should not be used. The uptake of water will accelerate the oxidation.

Analysis results

TEMED	
M.W.	116.21
Minimum assay	99%
Sulphate ash	<0.005%
Iron (Fe)	<0.00001%

Ordering information

Product	Use	Quantity	Code No.
TEMED	IEF, PAGE, Sequencing	25ml	17-1312-01

TEMED should be stored well sealed, dry and dark at room temperature.

Buffers

Tris

PlusOne quality Tris (Tris(hydroxymethyl)aminomethane) is dedicated for preparing electrophoresis buffers in the pH range 7.2–9.0. It is free from impurities that can disturb the polymerization.

Analysis results

Tris(hydroxymethyl)aminomethane	
M.W.	121.14
Minimum assay	99.8%
Melting point	170–171 °C
Water insoluble matter	0.003%
Sulphate	0.005%
Copper	0.0001%
Iron	0.0001%
Lead	0.0002%
Zinc	0.0001%

Ordering information

Product	Use	Quantity	Code No.
Tris	PAGE, Sequencing	500 g	17-1321-01

Tris should be stored at room temperature.

Boric Acid

Boric acid is used for making high quality electrophoresis buffers. PlusOne quality is free from impurities that disturb the polymerization of acrylamide and is specially suitable for preparing TBE buffer, the most frequently used buffer for DNA/RNA electrophoresis.

Analysis results

Ortho-boric acid	
M.W.	61.83
Minimum assay	99.8
Chromium	0.0001%
Cobalt	0.0001%
Copper	0.0002%
Iron	0.0002%
Lead	0.0002%
Magnesium	0.0005%
Manganese	0.0001%
Molybdenum	0.0001%
Nickel	0.0001%
Zinc	0.0005%

Ordering information

Product	Use	Quantity	Code No.
Boric acid	PAGE, Sequencing	500 g	17-1322-01

Boric acid should be stored at room temperature.

EDTA di-sodium salt

EDTA (Ethylene diamine tetra-acetic acid di-sodium salt di-hydrate) forms strong complexes with heavy metals such as copper and zinc. It is used in DNA electrophoresis buffers to inactivate nucleases by binding metal ions.

Analysis results

EDTA di-sodium salt di-hydrate	
M.W.	372.24
Minimum assay	99.5%
Calcium	0.002%
Copper	0.0001%
Iron	0.0005%
Lead	0.0005%
Magnesium	0.001%

Ordering information

Product	Use	Quantity	Code No.
EDTA, di-sodium salt	PAGE, Sequencing	100 g	17-1324-01

EDTA should be stored at room temperature.

Glycine

Glycine is frequently used as a component of electrophoresis buffers, especially in the Laemmli discontinuous buffer system.

Analysis results

Glycine	
M.W.	75.07
Minimum assay	99.0%
Copper	0.0001%
Iron	0.0001%
Lead	0.0002%

Ordering information

Product	Use	Quantity	Code No.
Glycine	PAGE, Sequencing	500 g	17-1323-01

Glycine should be stored at room temperature.

Barbitone-acetate Buffer

This pre-prepared buffer (pH=8.6, I=0.1) is a standard buffer for immunoelectrophoresis and serum separations. PlusOne buffer concentrate is accurately and reproducibly manufactured from high quality reagents and offers significant time saving and convenience. For cellulose acetate foils, 300 ml of the buffer is diluted to 500 ml with water. For agarose gel electrophoresis, 300 ml of the buffer is diluted to 525 ml with water. Other dilutions can also be used.

Contents per litre

Sodium acetate trihydrate	6.5 g
Sodium barbitone	8.87 g
Barbitone	1.13 g
Thiomersal	0.5 g

Ordering information

Product	Use	Quantity	Code No.
Barbitone-acetate buffer	Serum electrophoresis	1 000 ml	17-1333-01

Barbitone-acetate buffer should be stored at room temperature.

Non-barbitone Buffer

This non-barbitone buffer is used to replace barbitone buffers for immunoelectrophoresis and serum separations. It can be employed with both cellulose acetate foils and agarose gels. When used with cellulose acetate foils the separations obtained are virtually indistinguishable to those achieved with barbitone buffers. Recommended working solution is 1 part buffer and 9 parts of water. The pH of the working solution is 8.8.

Contents per litre

Tris(hydroxymethyl)aminomethane,	88 g
N-tris(hydroxymethyl) methyl glycine	28 g
p-Chlorophenyl acetic acid	24 g
Sodium chloride	17 g
Thiomersal	0.1 g

Ordering information

Product	Use	Quantity	Code No.
Non-barbitone buffer	Serum electrophoresis	250 ml	17-1334-01

Non-barbitone buffer should be stored at room temperature.

Additives and Sample Treatment

Urea

PlusOne quality urea is specially suitable for manual DNA sequencing, where separations are often run at high concentrations of urea. It is also used to improve the solubility of hydrophobic proteins close to their isoelectric points, especially in IEF.

Analysis results

Urea	
M.W.	60.06
Minimum assay	99.5%
Sulphate	0.001%
Copper	0.0001%
Iron	0.0001%
Lead	0.0002%
Biuret	0.05%

Ordering information

Product	Use	Quantity	Code No.
Urea	IEF, PAGE, Sequencing	500 g	17-1319-01

Urea should be stored dry at room temperature.

Formamide

A mild denaturant for nucleic acids with an action comparable to that of urea. Formamide works by disrupting hydrogen bonding. Gels with formamide can be used to estimate the number of base pairs in a nucleic acid.

Analysis results

Formamide	
M.W.	45.04
Minimum assay	99 %
Heavy metals (as Pb)	<0.0001%
DNase (exo- and endonucleases)	Not detected
RNases	Not detected
Phosphatases	Not detected
Proteases	Not detected

Ordering information

Product	Use	Quantity	Code No.
Formamide	IEF, PAGE, Sequencing	250 ml	17-1320-01

Formamide should be stored dry at room temperature.

Dithiothreitol

Dithiothreitol (DTT) at a concentration of 1% is used to reduce disulphide bonds for electrophoretic analysis of protein subunits. At low concentrations (1 mM) dithiothreitol is used to counteract the oxidation of proteins and preserve biological activity.

Analysis results

Dithiothreitol	
M.W.	154.24
Minimum assay (ex SH)	99%
Heavy metals (as Pb)	0.001
DNase (exo- and endonucleases)	Not detected
RNases	Not detected
Phosphatases	Not detected
Proteases	Not detected

Ordering information

Product	Use	Quantity	Code No.
Dithiothreitol	IEF,	1 g	17-1318-01
	SDS-PAGE	5 g	17-1318-02

DTT should be stored dry at +4 °C to +8 °C. Shelf life is 3 years.

Mercaptoethanol

Mercaptoethanol at a concentration of 1% is used to reduce disulphide bonds for electrophoretic analysis of protein subunits. At low concentrations (1 mM) mercaptoethanol is used to counteract the oxidation of proteins and preserve biological activity.

Analysis results

β-Mercaptoethanol	
M.W.	78.13
Minimum assay	99%
Weight per ml at 20 °C	1.12 g
Refractive index	1.499–1.502

Ordering information

Product	Use	Quantity	Code No.
Mercaptoethanol	IEF, SDS-PAGE	25 ml	17-1317-01

Mercaptoethanol should be stored dry at room temperature.

Glycerol

Glycerol is used as a stabilizing medium (increasing density and viscosity) when casting polyacrylamide gradients. In IEF, the pH gradient can be stabilized by introducing 10–15% glycerol. The increased viscosity reduces electroendosmosis and the extrusion of water onto the gel surface. PlusOne quality glycerol is free from impurities such as metals and antioxidants that inhibit polymerization.

Analysis results

Glycerol	
M.W.	92.09
Assay	86–88%
Density at 20 °C	1.224–1.230 g/ml
Refractive index at 20 °C	1.452–1.456
Copper	0.00005
Iron	0.00005%
Lead	0.00005%
Reducing substances	Not detected
Sugars (glucose)	0.004%

Ordering information

Product	Use	Quantity	Code No.
Glycerol 87%	IEF, PAGE, Sequencing	1 000 ml	17-1325-01

Glycerol should be stored at room temperature.

Detergents

Sodium dodecylsulphate (SDS)

The quality of SDS is of utmost importance for good electrophoretic results. Impurities with long carbon chains will influence micelle size and thus the migration of the proteins. A long carbon chain will also make staining difficult as impurities will adsorb to proteins and prevent binding of Coomassie.

Analysis results

SDS	
M.W.	288.38
Minimum assay (anhydrous material)	99%
Acidimetric	98.5%
C12 compounds by GLC	0.0001%
Phosphate	0.0002%
Lead	0.0002%

Ordering information

Product	Use	Quantity	Code No.
SDS	PAGE	100 g	17-1313-01

SDS should be stored at room temperature.

Triton X-100*

Triton X-100 (polyoxyethylene-p-t-octylphenol) is the most commonly used non-ionic detergent in IEF. In electrophoresis gels, concentrations from 0.05% to 0.5% are most common, while concentrations up to 2% are used in samples.

Analysis results

Triton X-100	
Active constituent	100%
Density at 20 °C	1.06

Ordering information

Product	Use	Quantity	Code No.
Triton X-100	IEF, PAGE	500 ml	17-1315-01

Triton X-100 should be stored dark at room temperature.
*Triton X-100 is a registered trade mark of Rohm & Haas Company.

CHAPS

CHAPS (3-((3-cholamidopropyl)dimethylammonio)-1-propanesulfonate) is used for protein solubilization during sample preparation for 2-D electrophoresis. CHAPS is a zwitterionic detergent with the ability to disrupt non-specific protein interactions and preserve the native form of proteins.

Ordering information

Product	Use	Quantity	Code No.
CHAPS	IEF, PAGE	1 g	17-1314-01

CHAPS should be stored dry at +4 °C to +8 °C

TWEEN 20**

Tween 20 (polyoxyethylene(20)sorbitan monolaurate) is a mild detergent for solubilization of proteins with preserved activity. It is used in PAGE, agarose electrophoresis and IEF. Tween 20 is a useful blocking agent when blotting onto nitrocellulose membranes. It can also be included in all washing buffers to prevent non-specific adsorption of protein to the membrane.

Analysis results

Tween 20	
Density at 20°C	1.1 g/ml
Hydroxyl number	97–113
Saponification number	40–51

Ordering information

Product	Use	Quantity	Code No.
Tween 20	IEF, PAGE	500 ml	17-1316-01

Tween 20 should be stored dark at room temperature.
**Tween 20 is a registered trademark of Atlas Chemical Industries Inc.

Stains

Ethidium Bromide

Widely used for detection of DNA and RNA, ethidium bromide binds to double-stranded regions to produce UV-fluorescent complexes. A band of as little as 50 nanograms of DNA can be detected with concentrations as low as 0.5 to 1 µg ethidium bromide per ml staining solution.

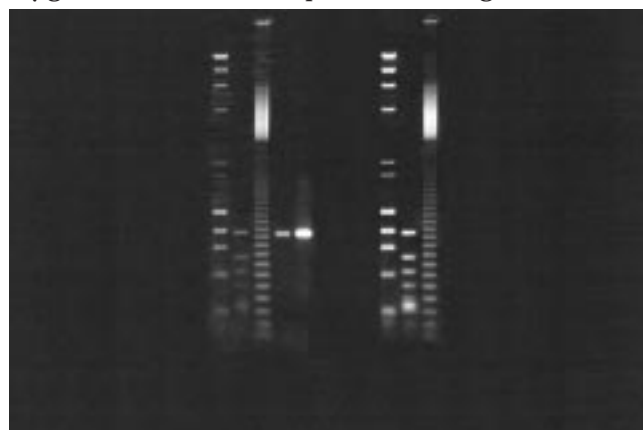


Fig. 5. Detection of DNA bands using ethidium bromide.

Analysis results

Ethidium bromide	
M.W.	394.32
Minimum assay	99%

Ordering information

Product	Use	Quantity	Code No.
Ethidium bromide solution 10 mg/ml	DNA/RNA detection	10 ml	17-1328-01

Ethidium bromide should be stored at room temperature.

Bromophenol Blue

Bromophenol Blue is the most popular tracking dye used in polyacrylamide gel electrophoresis in neutral or alkaline buffer systems.

Ordering information

Product	Use	Quantity	Code No.
Bromophenol Blue	IEF, PAGE, Sequencing	10 g	17-1329-01

Bromophenol Blue should be stored at room temperature.

Glass Plate Treatment

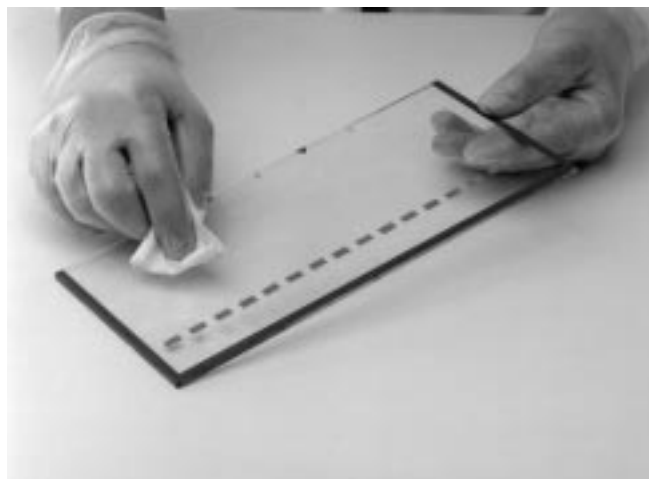


Fig. 6. Treatment of glass plates with Repel-Silane.

Repel-Silane ES

Repel-Silane (2% solution of dimethyldichlorosilane in octamethyl cyclotetrasiloxane, a low toxic Environmentally Safe solvent, free of ozone depleting properties) is used to prevent both polyacrylamide and agarose gels from sticking to glass surfaces in a gel casting mold. The casting mold is treated with Repel-Silane ES by painting the glass surfaces with the solution.

Ordering information

Product	Use	Quantity	Code No.
Repel-Silane ES	IEF, PAGE, Sequencing	500 ml	17-1332-01

Repel-Silane should be stored dark and dry at room temperature.

Bind-Silane

Bind-Silane (gamma-methacryloxy-propyl-trimethoxysilane) is used to covalently attach polyacrylamide gels to a glass surface. The gel will stay firmly attached to the glass during staining and drying procedures.

Ordering information

Product	Use	Quantity	Code No.
Bind-Silane	IEF, PAGE, Sequencing	25ml	17-1330-01

Bind-Silane should be stored dark and dry at room temperature. Shelf life is 3 years.

Other

DryStrip Cover Fluid

This specially purified fraction of mineral oil is used to protect Immobiline DryStrip from drying, or from other external interference, during the first dimension of 2-D electrophoresis.

Ordering information

Product	Use	Quantity	Code No.
DryStrip Cover Fluid	2-D Immobiline	1 000 ml	17-1335-01

DryStrip Cover Fluid should be stored dark at room temperature.

Amberlite* IRN-150L ion exchange resin

Amberlite IRN-150L ion exchange resin is a homogeneous mixture of strong cationic and anionic resins. Amberlite IRN-150L is used to remove trace ionic impurities from urea, Triton X-100, acrylamide or other non-ionic chemicals. Prior to use, the resin is added to the chemical solution to be purified and equilibrated for one hour before filtering.

Analysis results

Amberlite IRN-150L	
Ionic form	Hydrogen/Hydroxide
Shape	Spherical beads
Density	720g/l
Effective size	035-0.45 mm
Fines content	<0.5% <0.3 mm
Ionic capacity	>0.55 meq/ml

Ordering information

Product	Use	Quantity	Code No.
Amberlite IRN-150L	Purifying solution	500 g	17-1326-01

Amberlite ion exchange resin should be stored at room temperature.

* Amberlite is a registered trademark of Rohm & Haas Company.

