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Acrylamide, Chemzymes Ultra Pure®

High Purity, Low Conductivity, Electrophoresis Grade

INTRODUCTION

Acrylamide is a water-soluble monomer which is widely used in electrophoresis. Polyacrylamide gel electrophoresis (PAGE) is a versatile method for the separation, analysis, and characterization of nucleic acids, proteins and other charged species.[1, 2]

The optical clarity, physical strength, chemical purity, and reproducibility of the gels are highly dependent on the use of specially prepared and purified electrophoresis reagents. Polysciences is proud of its reputation for offering the finest ultra pure acrylamide that is used in key research laboratories throughout the world. Prepared by a proprietary method, Polysciences' acrylamide provides a lower conductivity and fewer contaminants then many acrylamides purified by recrystallization methods.

POLYMERIZATION OF ACRYLAMIDE

Electrophoresis gels are formed by the free radical polymerization of acrylamide with a crosslinker. Polymerization is achieved with an initiator (usually ammonium persulfate) and a catalyst, N,N,N',N'-tetramethylethylenediamine (TEMED). Polymerization may also be achieved photochemically with riboflavin and ultraviolet light replacing ammonium persulfate.

Polyacrylamide gels are sieve-like structures with pores, the dimensions of which are comparable to those of protein molecules. The pore size and handling characteristics of gels may be modified by various techniques to accomplish specific separations. The more commonly accepted variations include changing the ratio of acrylamide to water and the ratio of crosslinking agent to acrylamide. Various crosslinking agents and co-monomers may be used to produce the desired result. Through a combination of these variables, PAGE can separate proteins, nucleic acids, and related compounds according to both their size and surface charge.

For optimal PAGE, acrylamide with high purity, low conductivity, and high solubility is desired. Low acrylic acid content reduces unwanted chain termination during polymerization. Poor quality acrylamide with high levels of acrylic acid and linear polyacrylamide will copolymerize and can cause local pH changes in the gel. This can cause streaking and smearing of bands especially problematic in fluorescent DNA sequencing techniques.

The storage of acrylamide at room temperature, especially dissolved in water, will cause the breakdown of acrylamide into acrylic acid. This can be minimized by storing acry- lamide at 4°C and protected from light.

CROSSLINKING AGENTS

Crosslinking agents are used in the preparation of electrophoresis gels to control the water-swelling characteristics and pore size. The concentration of crosslinking agent can vary from approximately 2% to 25% based on acrylamide concentration. For example, the use of low levels of crosslinking agent (e.g., 0.3% based on acrylamide) in a gel consisting of about 10% total solid concentration seems to result in sharper bands and the resolution of wide molecular weight ranges.

A commonly used crosslinker in acrylamide gels for the separation of proteins is N,N'-methylenebisacrylamide (BIS). Bisacryamide yields "irreversible" gels. For separation techniques involving the recovery of bands such as quantitation by scintillation counting, it is desirable to have a method of solubilizing the gel under gentle conditions. For this purpose, crosslinking agents such as ethylene diacrylate[3] which dissolves when subjected to alkaline hydrolysis have been used. Other crosslinking agents include N,N'-diallyltartardiamide, N,N'-(1,2-dihydroxyethyl- ene) bisacrylamide, and N,N',N"-triallyl citric triamide which may be cleaved by mild periodic oxidation[4, 5]. Gels which are solubilizable by these techniques are called "reversible" gels.

PAGE FOR PROTEIN AND NUCLEIC ACID SEPARATIONS

SDS-PAGE is the most common method for separating proteins electrophoretically. When sodium dodecyl sulfate (SDS), a negatively charged detergent, is used as an additive in PAGE techniques, it binds to the proteins to help solubilize them. The SDS denatures the proteins and inparts a uniform negative charge. The shape of the protein is changed to a rod-like particle whose movement is now a function of size and molecular weight. Proteins ranging in size from 20,000 to 1,000,000 Da can be resolved with great accuracy.[6, 7]

SDS-PAGE gels can be run in continuous or discontinuous systems. In a continuous system, the gel is made with a single buffer at a single acrylamide concentration. In a discontinuous system, commonly used in

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Chemistry beyond the ordinary

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protein separations, the gel is composed of two layers - a stacking gel and resolving gel. The stacking gel, which is layered on top of the resolving gel, is composed of a lower acrylamide concentration. The function of the stacking gel is to focus and concentrate the proteins before they enter the resolving gel, resulting in higher resolution of the proteins.

PAGE is also used in a 2-Dimensional format for the separation of proteins, first by their isoelectric point (PI), then by their molecular weight. In the first dimension, an acrylamide gel containing ampholytes, small positively and negatively charged molecules, are used to separate proteins by isoelectric focusing (IEF). During the electrophoretic separation, the ampholytes set up a pH gradient. The proteins will migrate through the acrylamide gel until they reach the pH that is equal to their isoelectric point. In the second dimension, the proteins are separated by their molecular weight under SDS-PAGE conditions. With this method, over 1000 proteins can be resolved on a single SDS-PAGE gel.

Nucleic acids are also separated by PAGE. Typically, fragments of single standed DNA and RNA less than 1000bp are separated under denaturing PAGE conditions. The single base resolution of high concentration PAGE makes it especially useful for separating DNA sequencing reactions. In the case of nucleic acids, the denaturant used is urea.

CAUTION:

Acrylamide is a neurotoxin. It is readily absorbed through intact skin and can cause irritation of eyes. Avoid contact with eyes, skin, and clothing. Wear protective gloves, goggles, and clothing. Use only with adequate ventilation. Wash thoroughly with water after handling.

FIRST AID:

For large single ingestion, induce vomiting by giving syrup of ipecac in 30 mL, followed by 2 glasses of water. If ipecac is not available, touch the back of the throat with a spoon. Get medical attention. For eye contact, flush with copious amounts of water for 15 minutes and get medical attention. For skin contact, remove contaminated clothing and wash skin thoroughly. Wash clothing before reusing.

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Suggested Reading:

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TECHNICAL DATA SHEET 155

ORDERING INFORMATION:

Cat. #	Description	Size(s)
00019	Acrylamide, Chemzymes Ultra Pure®, 99.9%, Proprietary Purification Process MW 71.08, mp 84°, Insolubles: in H_2O & methanol - 0.005% max. Conductivity: < 2.0 micro mhos, max. (in 35% solution) Optical Density at 252nm: 1.35 max (7x10-3 molar) Free acrylic acid: 0.001% maximum pH (10% in 0.1M NaCl): 6.5 + 0.5	100g / 500g
17452	30%(w/v) Acrylamide/BIS Premix, 19:1, powder	30g / 6 x 30g
19847	30%(w/v) Acrylamide/BIS Premix, 29:1, powder	30g / 6 x 30g
17451	30%(w/v) Acrylamide/BIS Premix, 37.5:1, powder	30g / 6 x 30g
24170	40% (w/v) Acrylamide/BIS Premix, 19:1, solution	100ml / 6x100mL
24169	40% (w/v) Acrylamide/BIS Premix, 29:1, solution	100ml / 6x100mL
24165	40% (w/v) Acrylamide/BIS Premix, 37.5:1, solution	100ml / 6x100mL

ADDITIONAL REAGENTS:

Cat. #	Description	Size
00719	N, N'-Methylenebisacrylamide, Chemzymes Ultra Pure® (BIS)	25g / 100g
08036	N, N, N',N'-Tetramethylethylenediamine, Chemzymes Ultra Pure®, 99%	10 x 5mL amp
03945	Sodium dodecyl sulfate (Data Sheet #299)	100g / 1kg
24088	Tris-Glycine Buffer (TG Buffer) pH 8.3 ± 0.2, 10x Concentration	500mL / 1L
24089	Tris-Glycine Buffer (TG Buffer) pH 8.3 ± 0.2, 1x Powdered Blend	5pk / 10pk
24090	Tris-Glycine Buffer-SDS Buffer (TGS Buffer) pH 8.3 ± 0.2, 10x Concentration	500mL / 1L
04033	Ethidium Bromide	5g
04539	Acridine Orange, C.I. 46005, high purity	500mg / 5g
17052	Quinolinic phthalocyanine (Cuprolinic Blue)	100mg / 500mg
00352	Coomassie® Blue	10g / 50g / 100g
16717	Silver Stain Kit (for 25 slab gels) A simple, stable, controllable, and rapid method for detecting proteins in polyacrylamide slab gels. (Data Sheet #293)	1 kit

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