VIII. SPECIAL HANDLING INFORMATION

Ventilation and Engineering Controls: A local exhaust system which captures the contaminant at its source is recommended to prevent dispersion of the contaminant into the workroom air.

Respiratory Controls: For conditions of use where exposure to the dust is apparent, a NIOSH approved respirator may be worn. For emergencies, a self-contained breathing apparatus may be necessary.

Eye Protection: Use chemical safety goggles and/or a full face shield where splashing from solutions is possible. Contact lenses should not be worn when working with this material. Maintain eye wash fountain and quick drench facilities in the work area.

Gloves: Chemical resistant gloves such as latex.

Other Clothing and Equipment: Wear impervious protective clothing to prevent skin contact.

Work Practices, Hygienic Practices: Use good laboratory practices. Wash hands after using and before eating. Do not eat, drink, or smoke in the work area.

Other Handling and Storage Requirements: Maintain good housekeeping to control dust accumulations. Keep tightly sealed to protect quality. Store in a cool, dry, well ventilated place away from incompatible materials. An eye wash should be nearby and ready for use.

P
ctive
M
a
u
s
D u r
i
M a i n t
e n
c
o
C
a m i n
a t
E
u i p
ment: Clean-up personnel require protective clothing and a NIOSH approved respirator.

We believe that the information contained herein is current as of the date of this Material Safety Data. Since the use of this information and conditions of use of the product are not within the control of Bio-Rad Laboratories, it is the user's responsibility to

Bio-Rad Laboratories, 2000 Alfred Nobel Dr., Hercules, CA 94547

LIT300 Rev B

Piperazine di-Acrylamide (PDA)

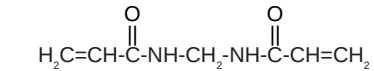
Catalog Number 161-0202

BIO-RAD

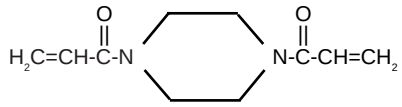
Introduction

PDA, a crosslinking agent which can be substituted for methylene-bis-acrylamide (bis) in polyacrylamide gels used for PAGE, IEF, and protein sequencing, offers several advantages for electrophoresis, particularly reduced background for silver staining, increased gel strength, and higher resolution gels (see Table 1). PDA can be substituted for bis without changing polymerization protocols.

PDA was originally developed to improve the sensitivity of silver stain detection in 2-D gels. In all detection methods, sensitivity depends on the signal-to-noise ratio. To increase this ratio, either the signal must be increased, or the noise must be decreased. Observations that basic and sulfur-containing amino acids are essential in the detection of peptides by the silver stain reaction[1-3] led to the hypothesis that amide groups of the methylene-bis-acrylamide crosslinker might be partially responsible for background noise in silver stained gels.[4] A series of experiments performed to test this hypothesis showed that PDA crosslinker (Figure 1) improves silver staining sensitivity. In addition to reducing silver staining background, the crosslinker has proved to have a wide range of beneficial effects on many polyacrylamide electrophoresis gels (Table 1).



N,N'-methylene-bis-acrylamide (bis)



Piperazine di-acrylamide (PDA)

Fig. 1. Bis has two amide groups separated by a single carbon. The piperazine ring of PDA eliminates the hydrogen atoms of the amide groups.

Table 1. Observed Advantages with PDA Crosslinked Polyacrylamide Gels

Application	Advantages of Substituting PDA for Bis
SDS-PAGE	• Increased gel strength with low %T gels
	• Reduced background in silver staining
2-D gels	• Increased gel strength simplifies tube gel handling
	• Tube gels can be stored for 1 month at 4 °C without precipitation of urea.
	• Reduced background in silver staining
	• Increased resolution of protein spots
Protein sequencing gels	• Decreased N-terminal blockage increases sensitivity of micro sequencing from 2-D blots

Use of PDA as a Crosslinker for Polyacrylamide Gels

The molecular weight of PDA is 194.23 while that of bis is 154.17. Although PDA is 1.26 times as heavy as bis, excellent electrophoresis results are obtained by substituting the crosslinker for bis on a gram for gram basis rather than on an equimolar basis. Table 2 can be used as a guide for formulation of separating gel and stacking gel solutions

The crosslinker concentration should be kept at or below 5% C, as higher concentrations turn the gel opaque. Experimental data (see Figure 2) indicate that as the concentration of crosslinker increases from 2 to 5% C, pore sizes in the gel decrease. Above 5% C there is an increase in pore size.

Polymerization conditions and catalyst concentrations are identical to current recommendations for bis. To start using PDA in your electrophoresis system, simply substitute PDA gram for gram for bis in making up your stock monomer solution. Use the monomer solution in the same way you use your current solution for casting the electrophoresis gels. It has been observed that PDA crosslinked gels shrink more than bis crosslinked gels when they are dried after equilibration in solutions containing only alcohol and acetic acid. Addition of 3-5% glycerol to the equilibration solution should overcome any shrinking problems.

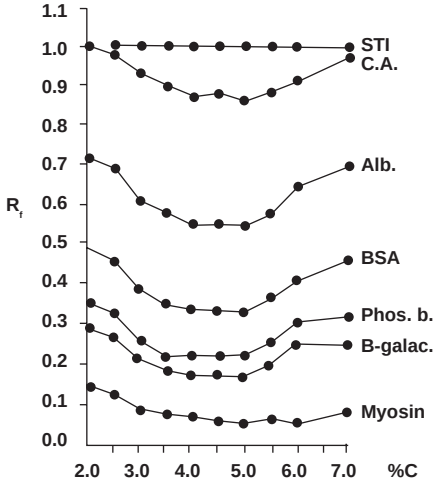


Fig. 2. Relative mobility measurements for PDA crosslinked gels. 10% SDS-PAGE gels with varying concentrations of crosslinker were used for SDS-PAGE electrophoresis of Bio-Rad's SDS-PAGE electrophoresis standards. As the crosslinker concentrations increase from 2–5% C, the relative mobility decreases. Above 5% C, pore size and mobility increase.

Table 2. Formulations for SDS-PAGE Separating and Stacking Gels

	Separating Gel (0.375 M Tris, pH 8.8)			Stacking Gel (0.125 M Tris, pH 6.8)
Monomer concentration (%T, 2.67% C)[a]	12%	7.5%	X%	4.0%
Acrylamide/PDA (30% T, 2.67% C stock)[b]	40.0 ml	25.0 ml	[c] ml	1.3 ml
Distilled water	33.5 ml	48.5 ml	[d] ml	6.1 ml
1.5 M Tris-HCl, pH 8.8	25.0 ml	25.0 ml	25.0 ml	-
0.5 M Tris-HCl, pH 6.8	-	-	-	2.5 ml
10% (w/v) SDS	1.0 ml	1.0 ml	1.0 ml	100 µl
10% ammonium persulfate (fresh)[e]	500 µl	500 µl	500 µl	50 µl
TEMED[e]	50 µl	50 µl	50 µl	10 µl[g]
Total[f]	100 ml	100 ml	100 ml	10 ml

a. The pore size of the polyacrylamide gel can be changed by adjusting either the total monomer concentration (%T) or by adjusting the crosslinking monomer concentration (%C). The most common method of changing the pore size is to adjust the %T. In diluting a stock solution, the %C remains constant.

$$\%T=[(g\ Acrylamide + g\ PDA) / Total\ Volume] \times 100$$
$$\%C=[g\ PDA/(g\ Acrylamide + g\ PDA)] \times 100$$

b. Acrylamide/PDA stock solution (30% T, 2.67% C) is prepared by dissolving 4.0 g of PDA and 146.0 g of acrylamide to 500 ml with distilled water. The solution is filtered and stored in the dark at 4 °C.

c. Calculate the volume of Acrylamide/PDA stock required for the desired total monomer concentration with the following formula: volume 30% T, 2.67% C stock = (X %T) x (3.33). For example, the volume of Acrylamide/PDA stock required to prepare 100 ml final volume of a 12% T monomer solution would be 40 ml: 12 x 3.33=40 ml.

d. Calculate the volume of water required for the desired total monomer concentration with the following formula: volume water = 73.5 - (volume 30% T, 2.67% C stock used). For example, the volume of water required to prepare 100 ml final volume of a 12% T monomer solution would be 33.5 ml: 73.5 - 40 = 33.5 ml.

e. Prepare the monomer solution by combining all reagents except ammonium persulfate and TEMED. Deaerate the solution under vacuum for at least 15 minutes. Add the two catalysts just prior to casting the gels.

f. You can prepare any desired volume of monomer solution by multiplying the 100 ml recipe with the desired multiplying factor.

g. Higher TEMED concentrations and faster polymerization are required for the stacking gel because of the inhibitory effect of atmospheric oxygen associated with the comb.

Stability and Handling

When stored at 4 °C in a dark, dry environment, PDA will be stable for at least 1 year. All PDA stock solutions should be stored at 4 °C. As with bis stock solutions, the most reproducible electrophoresis results will be obtained when the stock solution is filtered before storage and is not stored for more than 1 month.

Warning: Both bis and PDA are toxic. Avoid inhalation, contact with skin or eyes, or swallowing of these reagents. See the Material Safety Data (MSDS) section for further information.

References

1. Merril, C. R. and Pratt, M., *Anal. Biochem.*, **156**, 96-110 (1986).
2. Heukeshoven, J. and Dernick, R., *Electrophoresis*, **6**, 103-112 (1985).
3. Nielsen, B. L. and Brown, L .R., *Anal. Biochem.*, **144**, 311-315 (1984).
4. Hochstrasser, D., Patchornik, A. and Merril, C., *Anal. Biochem.*, **173**, 412-423 (1988).

Ordering Information

Catalog Number	Product Description
161-0202	PDA, 10 g
161-0101	Acrylamide, 500 g
161-0700	Ammonium Persulfate, 10 g
161-0800	TEMED, 5 ml

MATERIAL SAFETY DATA

I. PRODUCT IDENTIFICATION

Trade Name: Piperazine di-acrylamide
Catalog number: 161-0202
Chemical identity, Common names: PDA, diacrylyl-piperazine, PIP, 1,4-di-(1-oxo-2-propenyl) piperazine
Formula: $C_{10}H_{14}N_2O_2$ M.W.: 194.23

Manufacturers Name: **Emergency Telephone No:**

Bio-Rad Laboratories **(510) 741-1000**
2000 Alfred Nobel Drive
Hercules, CA 94547

Date Prepared or Revised: April 16, 1994
Name of Preparer: Thomas Slyker

II. HAZARDOUS INGREDIENTS

Chemical Names	CAS Number	Percent
1,4-di-(1-oxo-2-propenyl) piperazine	6342-17-2	100%

Exposure Limits in Air

ACGIH TLV	OSHA PEL	Other
No information found		*

* Acute oral LD50 values are unknown.

III. PHYSICAL/CHEMICAL CHARACTERISTICS

Boiling Point: N/A
Vapor Pressure: Not determined
Vapor Density (AIR=1): Unknown
Solubility in Water: Appreciable
Appearance and Color: Fine white crystals
Specific Gravity: (H_2O=1): Unknown
Melting Point: 95-96 °C
Evaporation Rate: (BUTYL ACETATE=1): N/A

IV. FIRE AND EXPLOSION HAZARD DATA

Flash Point: N/A **Flammable Limits:** N/A
(Method Used) N/A

Extinguishing Media: Water spray, dry chemical, alcohol foam, or carbon dioxide. Do not use high pressure water stream.

Special Fire Fighting Procedures: In the event of a fire, wear full protective clothing and NIOSH approved self-contained breathing apparatus with full facepiece operated in the pressure demand or other positive pressure mode.

Unusual Fire and Explosion Hazards: Dust may be explosive if mixed with air in critical proportions and in the presence of a source of ignition.

V. HEALTH HAZARD INFORMATION

Symptoms of Overexposure (for each potential route of exposure):

Inhaled: Unsaturated amides may cause irritation.

Contact with Skin or Eyes: Unsaturated amides may cause irritation.

Absorbed Through Skin: May be absorbed through skin.

Swallowed: Unsaturated amides may cause irritation.

Health Effects or Risks from Exposure

Acute: No specific information found. Irritant. Sax, fifth edition of Dangerous Properties of Industrial Materials, states that unsaturated amides are frequently an irritant and may be absorbed through the skin. In animal experiments, liver, kidney, and brain damage has been reported.

Chronic: No specific data found.

First Aid: Emergency Procedures

Eye Contact: Wash eyes with plenty of water for at least 15 minutes, lifting lower and upper eyelids occasionally. Get medical attention immediately.

Skin Contact: Remove any contaminated clothing. Wipe off excess from skin. Wash skin with plenty of water for at least 15 minutes. Get medical attention promptly.

Inhaled: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.

Swallowed: Induce vomiting immediately by giving two glasses of water and sticking finger down throat. Never give anything by mouth to an unconscious person. Call physician immediately.

Suspected Cancer Agent

__X__ No: This Product's Ingredients are Not Found in the Lists Below

Yes: _____Federal OSHA _____NTP _____IARC

Medical Conditions Aggravated by Exposure

Persons with pre-existing skin disorders, eye problems, or central or peripheral nervous system conditions may be more susceptible to the effects of the substance.

VI. REACTIVITY DATA

Stable __X__ Unstable _____

Conditions to Avoid: None known.

Incompatibility (Materials to avoid): Strong bases, strong acids, and oxidizing agents.

Hazardous Decomposition Products: Emits toxic fumes of nitrogen oxides and oxides of carbon when heated to decomposition.

Hazardous Polymerization May Occur __X__

Will Not Occur _____

Conditions to Avoid: Contact with oxidizing agents.

VII. SPILL, LEAK, AND DISPOSAL PROCEDURES

Spill Response Procedures: Sweep up dry material, flush the remainder with water, and decontaminate with a chemical cleaner.

Preparing Wastes for Disposal: Incineration or burial in approved landfill. Comply with all applicable federal, state, and local regulations on spill reporting, waste handling, and waste disposal.