Bio-Rad Protein Assay

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(1-800-424-6723)

BIO-RAD

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Section 1
Introduction

The Bio-Rad Protein Assay, based on the method of Bradford, is a simple and accurate procedure for determining concentration of solubilized protein. It involves the addition of an acidic dye to protein solution, and subsequent measurement at 595 nm with a spectrophotometer or microplate reader. Comparison to a standard curve provides a relative measurement of protein concentration.

1.1 Principle

The Bio-Rad Protein Assay is a dye-binding assay in which a differential color change of a dye occurs in response to various concentrations of protein.[1] The absorbance maximum for an acidic solution of Coomassie® Brilliant Blue G-250 dye shifts from 465 nm to 595 nm when binding to protein occurs.[2,3,4] The Coomassie blue dye binds to primarily basic and aromatic amino acid residues, especially arginine.[5] Spector[6] found that the extinction coefficient of a dye-albumin complex solution was constant over a 10-fold concentration range. Thus, Beer's law may be applied for accurate quantitation of protein by selecting an appropriate ratio of dye volume to sample concentration.

Interferences may be caused by chemical-protein and/or chemical-dye interactions. Table 1 lists those chemical reagents not directly affecting the development of dye color. (Note: Basic buffer conditions and detergents interfere with this assay.) Since every protein-chemical reagent combination has not been assayed, it is possible that some of the listed reagents produce interference in combination with certain proteins. However, with respect to proteins such as bovine serum albumin and gamma globulin, the listed reagents show little or no interference. The acceptable concentrations of reagents for the Standard Procedure are shown in Table 1. Equivalent concentrations of reagents for the Microassay Procedure (see Section 2) are 1/40 of those listed in this table, due to the difference of sample-to-dye ratios between the Standard and Microassay Procedures.

Table 1. Reagents Compatible with the Bio-Rad Protein Assay When Using the Standard Procedure.*

Acetate, 0.6 M
Acetone
Adenosine, 1 mM
Amino Acids
Ammonium sulfate, 1.0 M
Ampholytes, 0.5%
Acid pH
ATP, 1 mM
Barbital
BES, 2.5 M
Boric acid
Cacodylate-Tris, 0.1 M
CDTA, 0.05 M
Citrate, 0.05 M
Deoxycholate, 0.1%
Dithiothreitol, 1 M
DNA, 1 mg/ml
EDTA, 0.1 M
EGTA, 0.05 M
Ethanol
Eagle's MEM
Earle's salt solution
Formic acid, 1.0 M
Fructose
Glucose
Glutathione
Glycerol, 99%
Glycine, 0.1 M
Guanidine-HCl
Hank's salt solution
HEPES buffer, 0.1 M
KCl, 1.0 M
Malic acid, 0.2 M
$MgCl_2$, 1.0 M
Mercaptoethanol, 1.0 M
MES, 0.7 M
Methanol
MOPS, 0.2 M
NaCl, 5 M
NAD, 1 mM
NaSCN, 3 M
Peptones
Phenol, 5%
Phosphate, 1.0 M
PIPES, 0.5 M
Polyadenylic acid, 1 mM
Polypeptides (MW<3000)
Pyrophosphate, 0.2 M
rRNA, 0.25 mg/ml
tRNA, 0.4 mg/ml
total RNA, 0.30 mg/ml
SDS, 0.1%
Sodium phosphate
Streptomycin sulfate, 20%
Triton X-100, 0.1%
Tricine
Tyrosine, 1 mM
Thymidine, 1 mM
Tris, 2.0 M
Urea, 6 M
Vitamins

* Interference may be caused by chemical-protein and/or chemical-dye interactions. Table 1 lists those chemical reagents not directly affecting the development of dye color. Since every protein-chemical reagent combination has not been assayed, it is possible that some of the listed reagents produce interference in combination with certain proteins. However, with respect to proteins such as bovine albumin and globulin, the above listed reagents show little or no interference.

1.2 Product Description

Protein Assay Dye Reagent Concentrate (catalog number 500-0006) contains 450 ml of solution containing dye, phosphoric acid, and methanol. One bottle of dye reagent concentrate is sufficient for 450 assays using the standard assay procedure, or 2,250 assays using the microassay procedure.

The Dye Reagent Concentrate can be purchased in a kit with one of two standards: Bovine gamma globulin (Kit I, catalog number 500-0001) or bovine serum albumin (Kit II, catalog number 500-0002).

The Bio-Rad Protein Assay is for research use only.

1.3 Materials Required but Not Supplied

For standard assay

Spectrophotometer set to 595 nm

Cuvettes with 1 cm path length matched to laboratory spectrophotometer. Bio-Rad's disposable polystyrene cuvettes (catalog number 223-9950) are recommended

13 x 100 mm test tubes

Test tube rack for 13 x 100 mm test tubes

Vortex mixer

Whatman #1 filter (or equivalent) and funnel for dye reagent preparation

Graduated cylinders, pipets, and containers for reagent preparation and storage

Pipets accurately delivering 100 µl and 5.0 ml

For microplate assay

Microtiter plates

Pipets accurately delivering 200 µl and 800 µl

Section 2
Instructions

2.1 Reconstituting the Standard

To reconstitute the lyophilized bovine gamma globulin and bovine serum abumin standards, add 20 ml of deionized water and mix until dissolved. If the standard will not be used within 60 days, it should be aliquoted and frozen at -20 °C.

Note: The standards contain buffer salts required for solubilizing the protein.

2.2 Standard Procedure

1. Prepare dye reagent by diluting 1 part Dye Reagent Concentrate with 4 parts distilled, deionized (DDI) water. Filter through Whatman #1 filter (or equivalent) to remove particulates. This diluted reagent may be used for approximately 2 weeks when kept at room temperature.

2. Prepare three to five dilutions of a protein standard, which is representative of the protein solution to be tested. The linear range of the assay for BSA is 0.2 to 0.9 mg/ml, whereas with IgG the linear range is 0.2 to 1.5 mg/ml. (See Common Questions, question 4, for more information.)

3. Pipet 100 µl of each standard and sample solution into a clean, dry test tube. Protein solutions are normally assayed in duplicate or triplicate.

4. Add 5.0 ml of diluted dye reagent to each tube and vortex.

5. Incubate at room temperature for at least 5 minutes. Absorbance will increase over time; samples should incubate at room temperature for no more than 1 hour.

6. Measure absorbance at 595 nm.

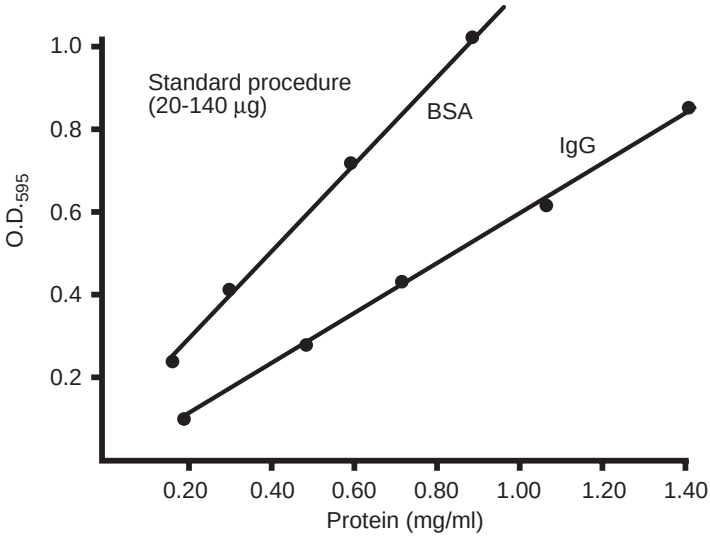

Fig. 1. Typical standard curve for the Bio-Rad Protein Assay, bovine gamma globulin (standard I), bovine serum albumin (standard II). $O.D._{595}$ corrected for blank - 200-1,400 µg/ml x 0.1 ml = 20-140 µg protein.

2.3 Microassay Procedure

1. Prepare three to five dilutions of a protein standard which is representative of the protein solution to be tested. The linear range of the assay for BSA is 1.2 to 10.0 µg/ml, whereas with IgG the linear range is 1.2 to 25 µg/ml. (See Common Questions, question 4, for more information.)

2. Pipet 800 µl of each standard and sample solution into a clean, dry test tube. Protein solutions are normally assayed in duplicate or triplicate.

3. Add 200 µl of dye reagent concentrate to each tube and vortex.

4. Incubate at room temperature for at least 5 minutes. Absorbance will increase over time; samples should incubate at room temperature for no more than 1 hour.

5. Measure absorbance at 595 nm.

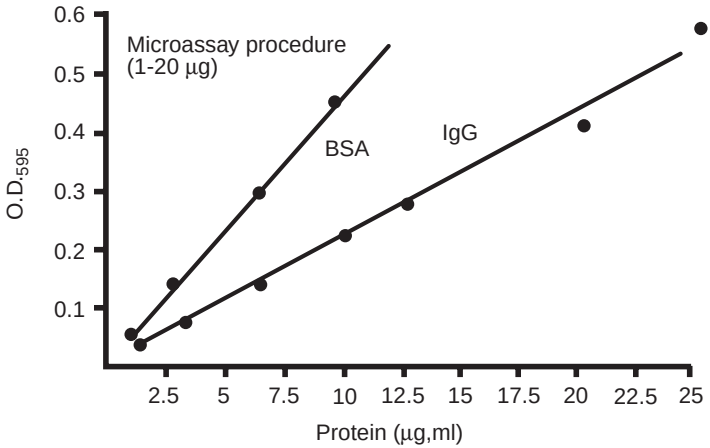

Fig. 2. Typical standard curve for the Bio-Rad Protein Microassay (1-20 µg/ml), bovine gamma globulin (standard I), bovine serum albumin (standard II).
$O.D._{595}$ corrected for blank. 1.25-25 µg/ml x 0.8 ml = 1-20 µg protein.

2.4 Microtiter Plate Protocols

The Bio-Rad Protein Assay can also be used with a microplate reader, such as Bio-Rad's Model 450 and 3550 Microplate Readers. The linear range of the Standard and Microassay procedures when used in the microtiter plate format is slightly changed, since the ratio of sample to dye is modified.

Standard Procedure for Microtiter Plates

1. Prepare dye reagent by diluting 1 part Dye Reagent Concentrate with 4 parts DDI water. Filter through a Whatman #1 filter (or equivalent) to remove particulates. This diluted reagent may be used for about 2 weeks when kept at room temperature.

2. Prepare three to five dilutions of a protein standard, which is representative of the protein solution to be tested. The linear range of this microtiter plate assay is 0.05 mg/ml to approximately 0.5 mg/ml. Protein solutions are normally assayed in duplicate or triplicate.

3. Pipet 10 µl of each standard and sample solution into separate microtiter plate wells.

4. Add 200 µl of diluted dye reagent to each well. Mix the sample and reagent thoroughly using a microplate mixer. Alternatively, use a multi-channel pipet to dispense the reagent. Depress the plunger repeatedly to mix the sample and reagent in the well. Replace with clean tips and add reagent to the next set of wells.

5. Incubate at room temperature for at least 5 minutes. Absorbance will increase over time; samples should incubate at room temperature for no more than 1 hour.

6. Measure absorbance at 595 nm.

Microassay Procedure for Microtiter Plates

1. Prepare three to five dilutions of a protein standard, which is representative of the protein solution to be tested. The linear range of the assay is 8.0 µg/ml to approximately 80 µg/ml. Protein solutions are normally assayed in duplicate or triplicate.

2. Pipet 160 µl of each standard and sample solution into separate microtiter plate wells.

3. Add 40 µl of dye reagent concentrate to each well. Mix the sample and reagent thoroughly using a microplate mixer. Alternatively, use a multi-channel pipet to dispense the reagent. Depress the plunger repeatedly to mix the sample and reagent in the well. Replace with clean tips and add reagent to the next set of wells.

4. Incubate at room temperature for at least 5 minutes. Absorbance will increase over time; samples should incubate at room temperature for no more than 1 hour.

5. Measure absorbance at 595 nm.

Section 3
Common Questions

1. The buffer that I normally use is not listed in the reagent compatibility list. How will I know if it interferes with the Bio-Rad Protein Assay?

   It is best to run two standard curves, one with protein in the same buffer as your sample and one with protein in water, and then plot a graph of protein concentration versus absorbance. If the buffer does not interfere, the two graphs of the standard curve will have identical slope. Partial interference can be compensated for by adding the buffer or interfering component to the standard curve for the actual protein assay.

2. My sample contains a detergent concentration which is incompatible with the Bio-Rad Protein Assay. How can I assay for protein?

   If the protein concentration is high enough, a sample with detergent can be diluted so that the concentration of detergent is reduced to 0.1% or less. Excess detergent can also be removed from a protein solution with Bio-Beads® SM-2 adsorbent.

   Alternatively, the Bio-Rad *DC* (detergent compatible) Protein Assay can be used. The *DC* Protein Assay is a modified Lowry assay which works in the presence of 1% ionic or nonionic detergent. This two-step method is ready to evaluate in just 15 minutes, and each kit will assay up to 500 samples with the standard method, or 10,000 samples with the microplate method.

3. Which protein assay method should I choose, the Bio-Rad (Bradford) Protein Assay or the *DC* (detergent compatible) Protein Assay?

   There are distinct advantages for each assay depending upon the application. The Bio-Rad Protein Assay, based on the Bradford method, can be used in the presence of sugars, DTT, and 2-mercaptoethanol which may interfere with the Bio-Rad *DC* Protein Assay. Alternatively, the Bio-Rad *DC* Protein Assay can be used in the presence of detergents and sodium hydroxide, two components known to interfere with the Bradford assay. If the sample is in a buffer that is compatible with both assays, then either may be used.

4. My sample is a mixture of proteins. Which protein standard should I use to generate the standard curve?

   In any protein assay, the best protein to use as a standard is a purified preparation of the protein being assayed. In the absence of such an absolute reference protein, one must select another protein as a **relative** standard. The best relative standard to use is one which has similar properties to, and which gives a color yield similar to that of, the protein(s) being assayed.

   Any purified protein can be selected as a reference standard if only *relative* protein values are desired. Bio-Rad offers two standards, Bovine gamma globulin (Standard I, catalog number 500-0005) and bovine serum albumin (Standard II, catalog number 500-0007).

5. How does the Bio-Rad Protein Assay compare to other assay methods?

   The Bio-Rad Protein Assay compares favorably with two other protein assay methods, the Biuret and the Lowry. In the table below, results are tabulated from dilutions made to gravimetrically prepared 10 mg/ml solutions of commercially available proteins. Bio-Rad Protein Standard I (bovine gamma globulin) was used for the Lowry and Bio-Rad Protein Assays, with bovine serum albumin was used for the Biuret method. All three assays display considerable variation in response to different proteins, but the averages were comparable.

Table 2. Comparison of Bio-Rad, Lowry, and Biuret Protein Assays for 23 Proteins

All assays were performed on appropriate dilutions of gravimetrically prepared 10 mg/ml solutions of commercially obtained proteins. Standards were as described in the text.

		Assay Results (Protein mg/ml)		
		Biuret	**Lowry**	**Bio-Rad**
1.	Alcohol dehydrogenase	5.8	5.0	7.8
2.	α-Amylase	6.8	6.0	8.3
3.	Bovine serum albumin	9.7	8.4	21.1
4.	Carbonic anhydrase	8.8	8.9	13.0
5.	Catalase	7.6	6.3	9.7
6.	α-Chymotrypsin	9.4	11.6	7.8
7.	Cytochrome c*	25.7	11.3	25.3
8.	Ovalbumin	10.2	10.1	9.4
9.	Fibrinogen	6.2	7.3	7.8
10.	Gamma globulin (rabbit)	9.4	11.8	8.0
11.	β-Galactosidase	9.5	9.9	7.9
12.	Hemoglobin (bovine)*	16.2	8.3	19.9
13.	Histones	9.7	9.2	15.8
14.	Hemocyanin	6.6	5.4	9.2
15.	Lysozyme	10.4	12.6	9.9
16.	Myoglobin*	13.7	7.9	20.7
17.	Ovomucoid	7.8	8.3	5.9
18.	Pepsin	9.8	12.4	4.1
19.	Ribonuclease	11.8	15.9	5.3
20.	Trypsin inhibitor (soy)	9.1	10.3	6.1
21.	Transferrin	8.5	9.0	12.6
22.	Trypsin	11.4	15.5	4.9
23.	Thyroglobulin	7.7	8.2	9.3
Average		10.1	9.5	10.9
*Average		8.8	9.6	9.2

* The second average eliminates from the figures the values for the three colored proteins shown by asterisks.

6. Is any sample preparation required?

   In general, no. However, the protein must be solubilized. (The sample can not be a suspension or an unfiltered homogenate.)

7. Can protein bound to a solid phase be quantitated?

   Yes, according to a recent reference in *Analytical Biochemistry*. Consult *Anal. Biochem.* **200**, 195 (1992) for additional information.

8. What is the shelf life of the dye reagent concentrate and the standards?

   The Dye Reagent Concentrate is stable for 1 year. For optimum shelf life, store at 4 ˚C.

   Lyophilized preparations of Protein Standard I (bovine gamma globulin) and Protein Standard II (bovine serum albumin), if included, should be refrigerated upon arrival. These lyophilized preparations have a shelf life of 1 year at 4 ˚C. Rehydrated and stored at 4 ˚C, the protein solutions should be used within 60 days. Rehydrated and stored in aliquots at -20 ˚C, the protein solutions should be used within 5 years. Avoid repeated freeze and thaw cycles of the protein standard.

9. Does the binding of the blue dye to cuvettes skew results?

   The amount of dye that binds to cuvettes, especially quartz cuvettes, is negligible.[1] Therefore, removal of the residual blue color between each sample reading is unnecessary. However, since the cuvettes may be used for subsequent procedures, there are several recommended treatments for dye removal:

   (a) Rinse cuvette with methanol, or

   (b) Rinse cuvette with concentrated glassware detergent, followed by DDI water, then acetone, or

   (c) Soak cuvette in 0.1 N HCl for a few hours, then wash as in (b).

   Bio-Rad's disposable polystyrene cuvettes (catalog number 223-9950) are recommended for the protein assay. They absorb less blue dye than glass cuvettes, and the cuvettes can be used as the mixing vessels for the standard assay if sample and reagent volumes are reduced to 50 µl and 2.50 ml, respectively. This technique eliminates the need for test tubes, and yields twice as many assays per bottle of reagent.

Section 4
Troubleshooting Guide

1. How can protein with a typical low dye response be assayed?

   Occasionally a protein will be assayed which yields exceptionally low color response to the Bio-Rad Protein Assay. One such protein is gelatin. Although the standard range (20-140 µg) is not detectable when the standard assay procedure is used, quantitation of the protein is possible when the sample-to-dye ratio is changed. By using the sample-to-dye ratio of the microassay procedure (800 µl sample + 200 µl dye reagent concentrate) a usable standard curve for moderate concentrations of gelatin is produced.

   Therefore, for a protein which yields exceptionally low color response to the Bio-Rad Protein Assay, quantitation in the standard range may be possible when the microassay sample-to-dye ratio is used.

   Other modifications of this dye binding assay to increase sensitivity have been reported.[7,8]

2. Absorbance of the protein solution is very low. What is the likely cause?

   The dye reagent concentrate may be old. If it is over 1 year old, replace with a new bottle of reagent.

   The sample may contain a substance which interferes with the reaction, such as detergent or sodium hydroxide. Check the compatibility guide (Table 1). Dilute the sample or switch to the *DC* Protein Assay if necessary.

   The molecular weight of the protein is low; the lower limit of detection for this method is approximately 3,000 to 5,000 daltons.

Section 5
Ordering Information

Catalog Number	Description
500-0001	**Bio-Rad Protein Assay Kit I,** includes 450 ml dye reagent concentrate and lyophilized bovine gamma globulin standard
500-0002	**Bio-Rad Protein Assay Kit II,** includes 450 ml dye reagent concentrate and lyophilized bovine serum albumin standard
500-0006	**Bio-Rad Protein Assay Dye Reagent Concentrate**, 450 ml
Related Materials	
500-0005	**Protein Standard I,** bovine gamma globulin, lyophilized. When reconstituted, it provides 20 ml of 1.4 ± 0.2 mg/ml solution.
500-0007	**Protein Standard II,** bovine serum albumin, lyophilized. When reconstituted, it provides 20 ml of 1.4 ± 0.2 mg/ml solution.
500-0116	**Bio-Rad *DC* Protein Assay Reagents Package,** includes 250 ml Reagent A, 2000 ml Reagent B, and 5 ml Reagent S.
500-0111	**Bio-Rad *DC* Protein Assay Kit I,** includes contents of Reagents Package (catalog 500-0116) and lyophilized bovine gamma globulin standard.
500-0112	**Bio-Rad *DC* Protein Assay Kit II,** includes contents of Reagents Package (catalog 500-0116) and lyophilized bovine serum albumin standard.
223-9950	**Disposable Polystyrene Cuvettes,** 100 - 3.5 ml cuvettes
223-9955	**Disposable Polystyrene Cuvettes,** 100 - 1.5 ml cuvettes
224-0096	**Costar 96 Well Flat Bottom EIA Plate,** polystyrene microtiter plates, 5 per package, carton of 100

MATERIAL SAFETY DATA SHEET

I. PRODUCT IDENTIFICATION

TRADE NAME: Methanol (as used in Protein Assay Kits I and II and Dye Reagent Concentrate)

Catalog No.: 500-0001, 500-0002, 500-0006

Chemical identity, Common names: Methanol; Methyl alcohol; Carbinol; Wood alcohol; Wood naphtha, Methyl hydroxide.

Formula: CH_3OH **M.W.:** 32.04

MANUFACTURER'S NAME:
BIO-RAD LABORATORIES
2000 ALFRED NOBEL DRIVE
HERCULES, CALIFORNIA 94547

EMERGENCY PHONE No:
510/741-1000

DATE PREPARED OR REVISED: March 24, 1994

NAME OF PREPARER: Roy Wood

II. HAZARDOUS INGREDIENTS

This product contains the following toxic chemical subject to the reporting requirements of section 313 of the Emergency Planning and Community Right-To-Know Act of 1986 and of 40 CFR 372:

Chemical	CAS		Exposure Limits in Air		
Names	Numbers	Percent*	ACGIH TLV	OSHA PEL	Other
Methanol	67-56-1	25%	200 ppm (TWA)	200 ppm (TWA)	ACGIH TLV 250 ppm (STEL)

LD50:5628 mg/kg oral-rat (RTECS)
TWA = 8-hour Time Weighted Average
STEL = Short Term Exposure Limit (15 minute)
* The other 75% of the mix is 25% water and 50% phosphoric acid [see MSDS #500-0001(b)].

III. PHYSICAL/CHEMICAL CHARACTERISTICS

BOILING POINT: 64.5 ˚C (148 ˚F)
VAPOR PRESSURE: 97 mm Hg at 20 ˚C
EVAPORATION RATE (BUTYL ACETATE = 1): 5.9
SOLUBILITY IN WATER: Complete
APPEARANCE AND COLOR: Clear liquid, alcohol odor.
SPECIFIC GRAVITY(H_2O = 1): 0.79
MELTING POINT: -98 °C (-144 ˚F)
VAPOR DENSITY(AIR = 1): 1.1

IV. FIRE AND EXPLOSION HAZARD DATA

FLASH POINT: 101 ˚F (for a 25% solution)
(METHOD USED): Closed Cup

FLAMMABLE LIMITS: LEL=6% UEL=36%

EXTINGUISHING MEDIA: Alcohol foam, carbon dioxide, dry chemical, or water fog.

SPECIAL FIRE FIGHTING PROCEDURES: Fire fighters should wear full protective clothing and self-contained breathing apparatus with full facepiece. Use water to keep fire-exposed containers cool.

UNUSUAL FIRE AND EXPLOSION HAZARDS: Vapor is heavier than air and may travel along the ground. Never use a welding or cutting torch near container (even empty). This material may burn with a flame which is invisible in daylight.

V. HEALTH HAZARD INFORMATION

SYMPTOMS OF OVEREXPOSURE (for each potential route of exposure):

INHALED: Excessive inhalation can cause nasal and respiratory irritation, visual disturbance, blurred vision, dizziness, giddiness, weakness, fatigue, nausea, vomiting, headache, possible unconsciousness, and asphyxiation.

CONTACT WITH SKIN OR EYES: Can cause irritation, tearing, blurred vision, drying of skin, and dermatitis.

ABSORBED THROUGH SKIN: May be absorbed through intact skin and produce systemic effects.

SWALLOWED: Can cause gastrointestinal irritation, headache, nausea, vomiting, blindness, unconsciousness, and death.

HEALTH EFFECTS OR RISKS FROM EXPOSURE

ACUTE: Poison! Swallowing or breathing high concentrations of methanol may produce headache, vomiting, nausea, irritation, dizziness, weakness, fatigue, giddiness, blurred vision, unconsciousness, blindness, and death. Methanol is extremely corrosive to the eyes.

Brief eye contact with the liquid or mist will severely damage the eyes and prolonged eye contact can cause permanent eye injury which may be followed by blindness.

Skin contact may irritate the skin, causing dermatitis. Methanol may be absorbed through intact skin, causing systemic effects.

CHRONIC: Marked impairment of vision, central nervous system damage, and death have been reported after prolonged or repeated exposure. Methyl alcohol may cause liver damage.

FIRST AID: EMERGENCY PROCEDURES

EYE CONTACT: Flush with large amounts of water for at least 15 minutes, lifting upper and lower lids occasionally. Get medical attention.

SKIN CONTACT: Flush skin with large amounts of water for at least 15 minutes, while removing contaminated clothing and shoes. Wash clothes before reuse. Get medical attention.

INHALED: Remove victim to fresh air. If breathing has stopped, give artificial respiration. Keep person warm. Get medical attention.

SWALLOWED: If conscious, immediately induce vomiting by giving two glasses of water and sticking a finger down the throat. After vomiting, give milk or water. Never give anything by mouth to an unconscious or convulsing person. Get medical attention 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

Those individuals with diseases of the eyes, liver, kidneys, and skin may be at increased risk from exposure.

VI. REACTIVITY DATA

STABLE _X_ **UNSTABLE**_____

CONDITIONS TO AVOID: Heat, sparks, and open flame.

INCOMPATIBILITY(Materials to avoid): Strong oxidizing agents, many metals, certain plastics, chloroform.

HAZARDOUS DECOMPOSITION PRODUCTS: Carbon oxides and formaldehyde may form when heated to decomposition.

HAZARDOUS POLYMERIZATION MAY OCCUR_____ WILL NOT OCCUR _X_

CONDITIONS TO AVOID: Heat, sparks, and open flame.

VII. SPILL, LEAK, AND DISPOSAL PROCEDURES

SPILL RESPONSE PROCEDURES: Dike and cover spill with non-combustible absorbent. Wear protective clothing including rubber apron, rubber gloves, rubber boots, chemical goggles, and face shield. Wear an appropriate NIOSH-approved respirator. Eliminate all ignition sources.

PREPARING WASTES FOR DISPOSAL: Comply with all applicable federal, state, and local regulations on spill reporting, waste handling, and waste disposal.

VIII. SPECIAL HANDLING INFORMATION

VENTILATION AND ENGINEERING CONTROLS: Provide sufficient mechanical explosion-proof ventilation to maintain exposure below exposure limits.

RESPIRATORY CONTROLS: Wear a NIOSH-approved respirator appropriate for the vapor concentration at the point of use. Appropriate respirators may be a supplied-air respirator or a self-contained breathing apparatus.

EYE PROTECTION: Chemical splash goggles or face shield.

GLOVES: Wear resistant gloves such as neoprene.

OTHER CLOTHING AND EQUIPMENT: Impervious clothing and boots.

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. Keep product away from heat, sparks, and flames.

OTHER HANDLING AND STORAGE REQUIREMENTS: Store in closed containers. All handling equipment should be electrically grounded. Store in a cool, dry, well-ventilated place away from incompatible materials. Store in a flammable liquid storage area or cabinet. An eye wash and safety shower should be nearby and ready for use.

PROTECTIVE MEASURES DURING MAINTENANCE OF CONTAMINATED EQUIPMENT: Protective clothing and appropriate respiratory protection should be worn. Do not cut, grind, weld, or drill on or near containers. Electrically ground all equipment and use only nonsparking tools.

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

MATERIAL SAFETY DATA SHEET

I. PRODUCT IDENTIFICATION

TRADE NAME: Phosphoric Acid (as used in Protein Assay Kits I & II and the Dye Reagent Concentrate).

Catalog No.: 500-000l, 500-0002, 500-0006

Chemical identity, Common names: Phosphoric acid, ortho-phosphoric acid, white phosphoric acid.

Formula: H_3PO_4 **M.W.:** 98.00

MANUFACTURER'S NAME:
BIO-RAD LABORATORIES
2000 ALFRED NOBEL DRIVE
HERCULES, CALIFORNIA 94547

EMERGENCY PHONE No:
510/741-1000

DATE PREPARED OR REVISED: March 31, 1993

NAME OF PREPARER: Roy Wood

II. HAZARDOUS INGREDIENTS

This product contains the following toxic chemical subject to the reporting requirements of section 313 of the Emergency Planning and Community Right-To-Know Act of 1986 and of 40 CFR 372:

Chemical	CAS		Exposure Limits in Air		
Names	Numbers	Percent*	ACGIH TLV	OSHA PEL	Other
Phosphoric acid	7664-38-2	50% (in mix)	1 mg/m^3 (TWA)	1 mg/m^3 (TWA)	ACGIH TLV 3 mg/m^3 (STEL)

TWA=8-hour Time Weighted Average

STEL=Short Term Exposure Limit (l5 minute)

* The other 50% of the mix is 25% water and 25% methanol [see MSDS #500-0001(a)].

III. PHYSICAL/CHEMICAL CHARACTERISTICS

BOILING POINT: l58 ˚C (3l6 ˚F)
VAPOR PRESSURE: 0.03 mm Hg at 20 ˚C
EVAPORATION RATE (BUTYL ACETATE = 1): N/A
SOLUBILITY IN WATER: Complete
APPEARANCE AND COLOR: Clear, colorless, syrupy liquid. Odorless.
SPECIFIC GRAVITY(H_2O = 1): l.69
MELTING POINT: 2l ˚C (70 ˚F)
VAPOR DENSITY(AIR = 1): 3.4

IV. FIRE AND EXPLOSION HAZARD DATA

FLASH POINT: N/A (not flammable) **FLAMMABLE LIMITS:** N/A
(METHOD USED): N/A

EXTINGUISHING MEDIA: Water fog or water spray

SPECIAL FIRE FIGHTING PROCEDURES: Water may be used to keep fire-exposed containers cool until fire is out. Fire fighters should wear full protective clothing and self-contained breathing apparatus with full facepiece.

UNUSUAL FIRE AND EXPLOSION HAZARDS: Phosphoric acid reacts with most metals to release hydrogen gas which can form explosive mixtures with air.

V. HEALTH HAZARD INFORMATION

SYMPTOMS OF OVEREXPOSURE (for each potential route of exposure):

INHALED: Irritation to nose, throat, and respiratory tract; coughing, chest pain, and difficulty in breathing.

CONTACT WITH SKIN OR EYES: Corrosive; may cause redness, burns, pain, blurred vision, severe irritation, and tissue damage.

ABSORBED THROUGH SKIN: Liquid can cause severe irritation and burns to the skin.

SWALLOWED: Corrosive; may cause sore throat, abdominal pain, nausea, and severe burns.

HEALTH EFFECTS OR RISKS FROM EXPOSURE

ACUTE: Phosphoric acid is extremely corrosive to the eyes, skin, nose, mouth, throat, and mucous membranes. Bronchitis, pulmonary edema, and chemical pneumonitis may occur with inhalation of vapors or mists. Breathing high concentrations may result in death. Brief eye contact with the liquid or mists will severely damage the eyes and prolonged contact may cause permanent eye injury which may be followed by blindness. Vapors will severely irritate skin.

Liquid and mists will severely burn the skin. Prolonged liquid contact will burn or destroy surrounding tissue and death may occur if burns extend over large portions of the body. Swallowing the liquid burns the tissues, causes severe abdominal pain, nausea, vomiting, and collapse. Swallowing large quantities can cause death.

CHRONIC: Skin contact may result in areas of destruction of skin tissue or primary irritant dermatitis. Similarly, inhalation of vapors or mists may cause damage to tissues and increase susceptibility to respiratory illness.

FIRST AID: EMERGENCY PROCEDURES

EYE CONTACT: Flush with large amounts of water for at least l5 minutes, lifting upper and lower lids occasionally. Get medical attention.

SKIN CONTACT: Flush skin with large amounts of water for at least l5 minutes, while removing contaminated clothing and shoes. Wash clothes before reuse. Get medical attention.

INHALED: Remove to fresh air. If not breathing, give artificial respiration. Get medical attention.

SWALLOWED: DO NOT induce vomiting. If conscious, give large quantities of milk or water. Never give anything by mouth to an unconscious or convulsing person. Get medical attention 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

History of respiratory or skin disease may increase risk from exposures.

VI. REACTIVITY DATA

STABLE _x_ **UNSTABLE**_____

CONDITIONS TO AVOID: Contact with metals can form hydrogen gas and be an explosion hazard.

INCOMPATIBILITY(Materials to avoid): Strong caustics, metals, cyanides, sulfides, and sulfites. A strong mineral acid, contact with water can cause heat generation and violent splattering.

HAZARDOUS DECOMPOSITION PRODUCTS: Toxic fumes of phosphorous oxides.

HAZARDOUS POLYMERIZATION MAY OCCUR_____ WILL NOT OCCUR _x_

CONDITIONS TO AVOID: N/A

VII. SPILL, LEAK, AND DISPOSAL PROCEDURES

SPILL RESPONSE PROCEDURES: Dike and cover spill with absorbent. Small spills can be carefully neutralized with sodium bicarbonate of lime. Wear protective clothing including an acid resistant suit, rubber gloves, rubber boots, chemical goggles, and face shield. Wear an appropriate NIOSH-approved respirator.

PREPARING WASTES FOR DISPOSAL: Comply with all applicable federal, state, and local regulations on spill reporting, waste handling, and waste disposal.

VIII. SPECIAL HANDLING INFORMATION

VENTILATION AND ENGINEERING CONTROLS: A local exhaust system is recommended to maintain levels below exposure limits.

RESPIRATORY CONTROLS: Wear a NIOSH-approved respirator appropriate for the vapor or mist concentration at the point of use. Appropriate respirators may be a full facepiece air-purifying respirator equipped with high efficiency cartridges, a supplied-air respirator, or a self-contained breathing apparatus.

EYE PROTECTION: Wear chemical safety goggles and a full face shield, where splashing is possible. Contact lenses should not be worn when working with this material.

GLOVES: Wear chemical resistant gloves such as neoprene, nitrile rubber, or natural rubber.

OTHER CLOTHING AND EQUIPMENT: Impervious protective clothing and boots.

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: Keep in tightly sealed container. Store in a cool, dry, well-ventilated place away from incompatible materials. Corrosive to mild steel. Store in rubber lined or 3l6 stainless steel. An eye wash and safety shower should be nearby and ready for use.

PROTECTIVE MEASURES DURING MAINTENANCE OF CONTAMINATED EQUIPMENT: Protective clothing and appropriate respiratory protection should be worn. Do not cut, grind, weld, or drill on or near containers.

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

Section 7
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