PRODUCT INFORMATION Horseradish Peroxidase Labeled Lectins

Catalog Number:	H-5101-1	Chemical Principle:	Peroxidase + $H_2O_2 \rightarrow$ Complex + AH_2 (don
Description:	Pure <i>Limax flavus</i> lectin (LFA) from garden slug, Horseradish Peroxidase conjugated.	Assay Reagents:	BUFFER: 0.01M Soc
			ENZYME: Dilute with
Lot Number: Protein	1 mg purified LFA Horseradish Peroxidas e / 1 ml Buffer.		DYE: 1% o-dianisid amber bottle or wrap
Concentration: (Based on OD280)	r ng pumleu Li'A noiseiaulsii reioxidase / r nii Bunei.		SUBSTRATE: Prepa water from stock concentration 0.0039
Carbohydrate Specificity:	Sialic acid (generic term for derivatives of N-Acetylneuraminic acid and N-Glycolylneuraminic acid.	Procedure:	1. Add 0.05 ml of Reaction test tub
Inhibitory Carbohydrate:	N-Acetylneuraminic acid and N-Glycolylneuraminic acid.		2. At time=0, add 1 100µl PBS to Co
Activity:	10-15 µg/ml will agglutinate type O human erythrocytes.		3. Measure and rec
Buffer:	0.05M Tris - 0.3M NaCl, pH 7.5.		seconds for 3 m minutes by stop NaN ₃ .
Chemical Used for Conjugation:	Horseradish Peroxidase.		4. Use this value to minute.
Storage:	Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze-thaw cycles. Clarify by centrifugation. No preservatives have been added. Sodium azide will inactivate the enzyme, peroxidase.	Enzyme Activity Calculations:	One unit of peroxida 1 µmole of peroxida absorbance of H ₂ O ₂ .
Stability:	The liquid material is stable for at least one year when stored frozen in aliquots.		$OD460 / min = \frac{O}{100}$
Caution:	Refer to the enclosed MSDS for information regarding lectins. The aluminum seals have sharp edges and the vial itself may have cracks which can cause		mg enzyme / ml rea
Procedure for Use:	lacerations. Use caution when opening the vial. See reverse side.		units / mg = $\frac{11.3}{11.3}$
Special Instructions:	 To Checking activity of Lectin HRP carbohydrate binding specificity. Dilute Lectin-HRP using PBS 100 times. (e.g. 10ul Lectin-HRP in 1ml PBS) Use Dilution from #1 for your application. (e.g. ELISA, Western Blot) After Lectin-Carbohydrate incubation, ensure use of PBS to wash 3 times to get rid of non-binding lectin-HRP or Free HRP, then add appropriate substrate for reaction. (i.e. 10ul TMB for dot blot.) Wait for 0 to 5 minutes for enzyme reaction to complete. 	Caution: Due to inhibitory sugar prese the Conjugate 50-100 times v	
References:	 Miller, R.L. (1987) Meth. Enzymol. 138 : 527-536. Ravindranath, M.H. and Paulson, J.C. (1987) Meth. Enzymol. 138 : 520-527. 		
	3. Roth, J., et al. (1985) Cell. 43 :287-295. 4. Biotechs: Sialic Acid and Related Products. Summer 1991 EV		

 Biotechs: Sialic Acid and Related Products. Summer 1991. EY Laboratories, Inc.

EY LABORATORIES, INC.

107 North Amphlett Blvd. San Mateo, CA 94401 Tel: 650-342-3296 Fax: 650-342-2648 Orders: 1-800-821-0044 (Outside CA only)

PRODUCT INFORMATION Horseradish Peroxidase Enzyme Activity Assay

Chemical Principle:	Peroxidase + $H_2O_2 \rightarrow Complex$ Complex + AH_2 (donor) \rightarrow Peroxidase + H_2O + A (colored)	
Assay Reagents:	BUFFER: 0.01M Sodium phosphate, pH 6.0.	
	ENZYME: Dilute with Buffer. Acceptable dilution: 1-2 μ g/ml.	
	DYE: 1% o-dianisidine in methanol prepared fresh daily. Store in amber bottle or wrapped in foil.	
	SUBSTRATE: Prepare 0.3% H_2O_2 solution in deionized or distilled water from stock H_2O_2 solution Prior to use dilute to a final concentration 0.003% in Buffer.	
Procedure:	1. Add 0.05 ml of DYE to 6.0 ml of SUBSTRATE. Add 2.9 ml to Reaction test tube and 2.9 ml to Control test tube.	
	 At time=0, add 100µl of diluted ENZYME to Reaction tube and 100µl PBS to Control tube. Mix thoroughly. 	
	3. Measure and record optical density at 460nm (OD460) every 15 seconds for 3 minutes, or take the end point reading after 3 minutes by stopping the reaction with $100 \mu l$ of concentrated NaN ₃ .	
	4. Use this value to determine the rate of change in absorbance per minute.	
Enzyme Activity Calculations:	One unit of peroxidase activity is that amount of enzyme decomposing 1 µmole of peroxide/minute at 25°C. 11.3 x 10^3 cm ⁻¹ is the molar absorbance of H ₂ O ₂ .	
	$OD460/min = \frac{OD460/Similar OD Control / Similar OD}{3minutes}$	
	mg enzyme / ml reaction mixture = $\frac{\text{[enzyme dilution]}}{30}$	
	units / mg = $\frac{OD460/\min}{}$	
	$11.3 \times \text{mg}$ enzyme / ml reaction mixture	

Caution: Due to inhibitory sugar present in the conjugates solution, to dilute the Conjugate 50-100 times with buffer before assay.



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PRODUCT IDENTIFICATION

- Name: Purified proteins or biotin labeled with Horseradish Peroxidase or Alkaline Phosphatase.
- Catalog HP-02, BA-104, BA-105, BA-108, BA-109, H-1102 to H-9000, LA-1104 to LA-Number (s): 9000, PA-2100 to PA-2701, AA-2100 to AA-2701, HAF-001 to HAF-2354, AAF-001 to AAF-2354, HA-01 to HA-013, AA-01 to AA-013, HAL-1104 to HAL-4701, AAL-1104 to AAL-4701.
- Synonyms: Protein A, Avidin (egg white), Biotin, Lectins, Secondary Antibodies labeled with Horseradish Peroxidase or Alkaline Phosphatase.

EMERGENCY INFORMATION

EY Laboratories, Inc.	EMERGENCY PHONE:	
107 North Amphlett Blvd.	650 342 3296	
San Mateo, CA 94401	050 542 5250	

HAZARDOUS COMPONENTS

Specific protein(s) as listed on the vial label. Solutions are at a concentration generally greater than 0.5mg protein/ ml. Biological activity of these labeled proteins will vary. Horseradish Peroxidase and Alkaline Phosphatase are both potent enzymes which may be harmful if ingested, inhaled, or allowed to absorb through the skin. Both enzymes are known to cause allergic responses in sensitive individuals.

HEALTH HAZARD INFORMATION

EXPOSURE LIMITS:	None established. The toxicological properties of these products have not	
	been thoroughly investigated. Care should be taken when handling any of	
	these materials.	
EFFECTS OF OVEREXPOSURE: ROUTES OF EXPOSURE:	May causes localized eye, skin, or mucous membrane irritation. Some sensitive individuals may develop a chronic allergic reaction with exposure. Inhalation of powders and skin contact with liquids are the primary routes of exposure. Care should be taken to avoid the formation of aerosols when handling any of the solutions.	

PHYSICAL CHARACTERISTICS

APPEARANCE: SOLUBILITY:

PRECAUTIONS:

NOTE:

Powders are a light brown. Solutions will be light to dark brown. Powders are completely soluble in many biological buffers and water. All liquids are completely miscible in water and biological buffers.

FIRE AND EXPLOSION HAZARDS EXTINGUISHING MEDIA: SPECIAL FIRE FIGHTING

Not considered to be a fire hazard. Water spray or CO₂. None required.

Alkaline Phosphatase conjugates contain less than 0.05% sodium azide as a preservative. Azide may react with lead and copper plumbing to form explosive metal azides. Flush with copious amounts of water when disposing material in the sink.

MSDS for Horseradish or Alkaline Phosphatase Labeled Proteins & Biotin Continued - page 2 of 2.

REACTIVITY DATA

STABILITY:

HAZARDOUS POLYMERIZATION:	
INCOMPATIBILITY:	

Stable. The nature of any decomposition products are not known. They are not believed to be hazardous. Will NOT occur. None known. (Lead and copper may react with sodium azide).

SPILL / LEAK PROCEDURES

MATERIAL RELEASE / SPILL:	Avoid contact with powder or liquid. Clean up spill with a paper towel soaked in household bleach. Do not allow solutions to dry on environmental surfaces. Wash affected area with detergent after the area
	has been treated with bleach.
WASTE DISPOSAL:	Incinerate, autoclave, or dispose of paper waste in accordance with all
	Local, State, and Federal regulations. Due to the small quantities of
	material involved these products are generally not considered to be environmental hazards. All of these proteins are fully biodegradable.

EMERGENCY FIRST AID PROCEDURES

May be harmful if swallowed, inhaled, or allowed to absorb through the skin. Wash contacted area with water for 15 minutes. If inhaled remove to fresh air. Report exposure to the appropriate safety official. Consult a physician if irritation occurs or if there is any indication of an allergic response, such as watering eyes, sneezing, or difficulty breathing. Any eye contact should be reported to a physician immediately

SPECIAL HANDLING PRECAUTIONS

VENTILATION:	No special ventilation is required but it is recommended to
EYE PROTECTION:	handle these reagents in a fume hood when possible. Required. Goggles or safety glasses with a side shield are recommended.
RESPIRATORY PROTECTION:	Recommended as a safety precaution, specifically when working with powders. An approved respirator may be required for those individuals already known to be
PROTECTIVE GLOVES:	sensitive to these materials. Required when handling any of these materials.

SPECIAL PRECAUTIONS

This material is for research and experimental application only. It is not intended for food, drug, household, agricultural, or cosmetic use. All materials should be handled only by technically qualified individuals experienced with working with potentially hazardous chemicals. The above information is correct to the best of our knowledge. The user should make independent decisions regarding completeness of the information, based on all sources available. EY Laboratories, Inc. shall not be held liable for any damage resulting from handling or contact with the above product.



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