PRODUCT INFORMATION
Horseradish Peroxidase Labeled Lectins

Catalog Number: H-2104-1

Description: Pure *Triticum vulgare* lectin (WGA) from wheat germ, Horseradish Peroxidase conjugated. Designed for trans-synaptic neuron transport.

Lot Number:

Protein Concentration: (Based on OD 280) 1 mg purified WGA Horseradish Peroxidase / vial. Reconstitute with 0.04 ml sterile distilled water for a final protein concentration of 25mg/ml (2.5%) in 0.01M Phosphate - 0.15M NaCl, pH 7.45. This concentration of the lectin has been used for iontophoresis and neuron transport.

Carbohydrate Specificity: $(GlcNAc\text{-}\beta\text{-}(1,4)\text{-}GlcNAc)_{1\text{-}4}$>β-GlcNAc>Neu5Ac.

Inhibitory Carbohydrate: GlcNAc β(1,4) GlcNAc β(1,4) GlcNAc>GlcNAc β(1,4) GlcNAc> GlcNAc>>sialic acid(Neu5Ac)>>GalNAc.

Activity: Less than 4mg/ml will agglutinate human type O erythrocytes. Less than 1 µg/ml will agglutinate neuraminidase treated erythrocytes.

Buffer: 0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4.

Chemical Used for Conjugation: Horseradish Peroxidase.

Storage: Store lyophilized powder refrigerated at 5 - 8°C or frozen. Store liquid frozen in aliquots. Avoid freeze-thaw cycles.

Stability: The liquid material is stable for at least one year when stored frozen in aliquots.

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 lacerations. Use caution when opening the vial.

Procedure for Use: See reverse side.

References:

1. Peters, B.P., et al. (1979) Biochemistry. **18** : 5505-5511.
2. Lotan, R. et. al. (1975) Biochem. Biophys. Res. Comm. **62** : 144-150.
3. Ebisu, S., et al. (1977) Carbohydrate Res. **58** : 187-191.
4. Watanabe, K. and Hakomori, S. -I. (1973) FEBS Lett. **37** : 317-320.
5. Yamamoto, K., et al. (1981) Biochemistry. **20** : 5894-5899.

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(Outside CA only)

PRODUCT INFORMATION
Horseradish Peroxidase Enzyme Activity Assay

Chemical Principle:
Peroxidase + H_2O_2 → Complex
Complex + AH_2 (donor) → Peroxidase + H_2O + A (colored)

Assay Reagents:

BUFFER: 0.01M Sodium phosphate, pH 6.0.

ENZYME: Dilute with Buffer. Acceptable dilution: 1-2 µg/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.
2. 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 100µl 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 25ºC. $11.3 \times 10^3\ cm^{-1}$ is the molar absorbance of H_2O_2.

$$\text{OD460 / min} = \frac{\text{OD460 / 3min - OD Control / 3minutes}}{\text{3minutes}}$$

$$\text{mg enzyme / ml reaction mixture} = \frac{\text{[enzyme dilution]}}{30}$$

$$\text{units / mg} = \frac{\text{OD460 / min}}{11.3 \times \text{mg enzyme / ml reaction mixture}}$$

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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MATERIAL SAFETY DATA SHEET

Effective Date: March 31, 2006
Revision 4

PRODUCT IDENTIFICATION

Name: Purified proteins or biotin labeled with Horseradish Peroxidase or Alkaline Phosphatase.

Catalog Number (s): HP-02, BA-104, BA-105, BA-108, BA-109, H-1102 to H-9000, LA-1104 to LA-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.
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EMERGENCY PHONE:
650 342 3296

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: May causes localized eye, skin, or mucous membrane irritation. Some sensitive individuals may develop a chronic allergic reaction with exposure.

ROUTES OF 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: Powders are a light brown. Solutions will be light to dark brown.

SOLUBILITY: Powders are completely soluble in many biological buffers and water. All liquids are completely miscible in water and biological buffers.

FIRE AND EXPLOSION HAZARDS

Not considered to be a fire hazard.

EXTINGUISHING MEDIA: Water spray or CO_2.

SPECIAL FIRE FIGHTING PRECAUTIONS: None required.

NOTE: 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.

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REACTIVITY DATA

STABILITY: Stable. The nature of any decomposition products are not known. They are not believed to be hazardous.

HAZARDOUS POLYMERIZATION: Will NOT occur.

INCOMPATIBILITY: 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 handle these reagents in a fume hood when possible.

EYE PROTECTION: 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 sensitive to these materials.

PROTECTIVE GLOVES: 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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