PRODUCT INFORMATION
TRITC Labeled Lectin

Catalog Number: R-1501-1

Description: Pure *Limulus polyphemus* lectin (LPA) from horseshoe crab, TRITC conjugated.

Lot Number:

Protein Concentration: (Based on OD 280) 1 mg purified LPA TRITC / 1 ml Buffer.

TRITC / Protein Ratio: (OD 550 / OD 280)

Purification Procedure: Gel filtration performed after conjugation to remove free TRITC.

Carbohydrate Specificity: Sialic Acid (N-Acetyl neuraminic acid).

Inhibitory Carbohydrate: N-Acetylneuraminic acid and N-Glycolylneuraminic acid.

Activity: 10-20 µg/ml will agglutinate type O human erythrocytes. As much as 100 µg/ml may be necessary to agglutinate type A or B cells.

Buffer: 0.05M Tris - 0.15M NaCl, 0.01M $CaCl_2$, pH 8.0. Contains 0.05% sodium azide as a preservative.

Chemical Used for Conjugation: Tetramethylrhodamine Isothiocyanate, TRITC.

Storage: Store liquid material refrigerated in aliquots in amber vials or covered with foil. DO NOT FREEZE.

Stability: The liquid material is stable for at least 1 year when stored refrigerated in aliquots with 0.05% sodium azide added as a preservative.

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.

Remarks: Calcium is REQUIRED for binding. The addition of millimolar concentrations of sialic acid in the above buffer of the addition of a calcium chelting agent such as EDTA may be used to inhibit binding. LPA is composed if 18-20 noncovalently bound subunits and may precipitate if frozen. Clarify by low speed centrifugation.

Fluorescent Conjugates are extremely light sensitive.

References:

1. Roche, A. C., et.al. (1974) Biochem. Biophys. Acta. **371**:242-254.
2. Marchalonis, et.al. (1968) J.Mol.Biol.**32**:453.
3. Roche, A., et.al. (1975) FEBS Lett. **57**:245.
4. Lotan, R., et al. (1977) Biochem. 16, **9**:1787.
5. Robey, F.A. et.al. (1981) J. Biol. Chem. **256**:969-975.
6. Pardoe, G.I., et.al. (1970) Immunol. **18**:73-83.

Sample Only

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)

General Procedure
Fluorescent Labeled Lectin

The following is a general Procedure and Trouble-Shooting Guide. The information is provided only for your convenience. The success of your experiments are not guaranteed by EY Laboratories, Inc.

Tissue Sections

1. Wash and block tissue section. Do not use serum products, they contain glycoproteins which may lead to high levels of non specific background. After blocking, rinse briefly with Buffer (See reverse side).
2. Dilute **Fluorescent Labeled Lectin** to desired concentration 20-100 µg/ml using Buffer.
3. Incubate tissue section with Fluorescent Labeled Lectin for 30 minutes in a moist chamber.
4. Wash tissue section with Buffer three times.
5. Examine tissue section with Fluorescent microscope. Use appropriate filter.
   Ref. M. Immbar et. al., (1973). Intnl. Journal of Cancer, **12**, 93-99

Cell Suspension

1. Wash cells with Buffer (See reverse side.)
2. Collect cells by centrifugation.
3. Dilute **Fluorescent Labeled Lectin** to 100 µg/ml using Buffer.
4. Incubate approximately $1x10^6$ cells with 1 ml diluted Fluorescent labeled Lectin for 15 minutes at room temperature or in a 37°C water bath.
5. Wash cells with Buffer three times using centrifugation.
6. Examine cells, with or without fixation with Fluorescent microscope. Use appropriate filter.
   Ref. K. Phiss. (1977). Experimental Pathology, **14**, S15

Fluorochromes must be protected from light. Perform incubation, when practical, in a dark room or covered in foil.

Absorption and Emission

	Absorption/Excitation Rate	Emission Max.
FITC	492 nm	517 nm
TRITC	554 nm	570 nm
Texas Red™	596 nm	615 nm

Carbohydrate Inhibition

Inhibition of lectin binding may be accomplished by using one of two procedures:

A. Before incubating with **Fluorescent Labeled Lectin**, incubate section or cells with inhibitory carbohydrate for 30-60 minutes at room temperature. NOTE: Complete inhibition may NOT occur.
B. Preincubate diluted **Fluorescent Labeled Lectin** with inhibitory carbohydrate for 30-60 minutes at room temperature before applying to section or cells.

TROUBLE SHOOTING GUIDE

Problem	Cause	Solution
Weak or no Staining	1. Low concentration of specific oligosaccharide on sample. 2. Low concentration of lectin conjugate. 3. Insufficient incubation time. 4. Photobleaching	Causes #1 - #3 a. Increase incubation time. b. Increase concentration conjugate. a. Avoid exposure to light.
High Background	1. Lectin conjugate is too concentrated. 2. Insufficient washing. 3. Autofluorescent sample.	a. Decrease concentration of Lectin conjugate. b. Shorten incubation times. a. Perform multiple washings and prolong washing time. a. Use fluorochrome with different excitation and emission spectrum. b. Use a different lectin conjugate (enzyme or colloidal gold).
Unexpected Staining Pattern	Multiple causes	a. Perform control reactions. b. Use other cytochemical technique to prove or disprove the findings.

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MATERIAL SAFETY DATA SHEET

Effective Date: March 31, 2006
Revision 4

PRODUCT IDENTIFICATION

Name:	Purified proteins labeled with fluorescein isothiocyanate (FITC), tetramethylrhodamine isothiocyanate (TRITC), or Texas Red a trademark of Molecular Probes for the sulfonyl chloride derivative of sulforhodamine 101
Catalog Number (s):	FP-01, RP-01, TP-01, F-1102 to F-9000, R-1102 to R-9000,T-1102 to T-9000, FA-2100 to FA-2701, RA-2100 to RA-2701, TA-2100 to TA-2701, FAF-001 to FAF-2354, RAF-001 to RAF-2354, TAF-001 to TAF-2354, FAL-1104 to FAL-4701, RAL-1104 to RAL-4701, TAL-1104 to TAL-4701, FA-01 to FA-013, TA-01 to TA-013, DM1011F to DM1064F, FNP-01 to FNP-05,BA-101, BA-102, BA-612.
Synonyms:	Protein A, Avidin (egg white), Glycosylated Bovine Serum Albumin, Lectins, Secondary and Monoclonal Antibodies labeled with FITC, TRITC, or Texas Red®

EMERGENCY INFORMATION

EY Laboratories, Inc.
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EMERGENCY PHONE:
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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. FITC, TRITC, and Texas Red® are possible carcinogens in their pure form. Compounds with similar chemical structures are known to be reactive with proteins and other biomolecules. The complete properties of the dyes after labeling have not been evaluated. These compounds should be treated as potentially hazardous. All solutions contain less than 0.05% sodium azide as a preservative.

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:	Causes localized eye, skin, or mucous membrane irritation. Some sensitive individuals may develop a chronic allergic reaction with exposure. The known effects are due to the protein. No specific effects of the bound dye are known at this time.
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 orange. Solutions will be yellow to dark purple.
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. At high concentrations the chemicals may emit toxic fumes. Such high concentrations are not normally found in a research laboratory.

EXTINGUISHING MEDIA:	Dry chemical powder or CO_2.
SPECIAL FIRE FIGHTING PRECAUTIONS:	Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

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NOTE: Most solutions 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.

REACTIVITY DATA

STABILITY:	Stable. Decomposition products are not known to be hazardous.
HAZARDOUS POLYMERIZATION:	Will NOT occur.
INCOMPATIBILITY:	Alcohols, strong bases and acids, strong oxidizing agents, and heat. (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 environment al 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.

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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