### **PRODUCT INFORMATION TRITC Labeled Lectin**

Catalog Number:	R-7001-1		
Description:	Pure Lycopersicon esculentum lectin (LEA) from Tomato, TRITC conjugated.		
Lot Number:			
Protein Concentration: (Based on OD 280)	1 mg purified LEA TRITC / 1 ml Buffer.		
TRITC / Protein Ratio: (OD 550 / OD 280)			
Purification Procedure:	Gel filtration performed after conjugation to remove free TRITC.		
Carbohydrate Specificity:	$\beta$ (1,4)-linked N-Acetylglucosamine.		
Inhibitory Carbohydrate:	GlcNAc $\beta(1,4)$ GlcNAc oligomers up to 4 carbohydrate units. The GlcNAc residues do not need to appear consecutively.		
Activity:	50-60 $\mu$ g/ml will agglutinate type O human erythrocytes. 5-10 $\mu$ g/ml will agglutinate neuraminidase treated cells.		
Buffer:	0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4. Contains 0.05% sodium azide as a preservative.		
Chemical Used for Conjugation:	Tetramethylrhodamine Isothiocyanate, TRITC.		
Storage:	Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze thaw cycles. Clarify by centrifugation.		
Stability:	The liquid material is stable for at least 1 year when stored frozen 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:	Fluorescent Conjugates are extremely light sensitive.		
References:	<ol> <li>Nachbar, M. S., et al. (1980) J. Biol. Chem. 255 : 2056-2061.</li> <li>Kilpatrick, D. C., et al. (1983) Anal. Biochem. 134 : 205-209.</li> <li>Slifkin, M. and Cumbie, R. (1987) J. Clin. Micro. 25 : 1172.</li> </ol>		

**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** 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).

<ol> <li>Dilute Fluorescent Labeled Lectin to desired concentration 20-100 μg/ml using Buffer.</li> <li>Incubate tissue section with Fluorescent Labeled Lectin for 30 minutes in a moist chamber.</li> <li>Wash tissue section with Buffer three times.</li> <li>Examine tissue section with Fluorescent microscope. Use appropriate filter. Ref. M. Immbar et. al., (1973). Inthl. Journal of Cancer, 12, 93-99         <ul> <li>Cell Suspension</li> <li>Wash cells with Buffer (See reverse side.)</li> <li>Collect cells by centrifugation.</li> <li>Dilute Fluorescent Labeled Lectin to 100 μg/ml using Buffer.</li> <li>Incubate approximately 1x 10<sup>6</sup> cells with 1 ml diluted Fluorescent labeled Lectin for 15 minutes at room</li> </ul> </li> </ol>
<ol> <li>Wash tissue section with Buffer three times.</li> <li>Examine tissue section with Fluorescent microscope. Use appropriate filter. Ref. M. Immbar et. al., (1973). Intnl. Journal of Cancer, 12, 93-99         Cell Suspension     </li> <li>Wash cells with Buffer (See reverse side.)</li> <li>Collect cells by centrifugation.</li> <li>Dilute Fluorescent Labeled Lectin to 100 µg/ml using Buffer.</li> </ol>
<ol> <li>Examine tissue section with Fluorescent microscope. Use appropriate filter. Ref. M. Immbar et. al., (1973). Intnl. Journal of Cancer, 12, 93-99         Cell Suspension     </li> <li>Wash cells with Buffer (See reverse side.)</li> <li>Collect cells by centrifugation.</li> <li>Dilute Fluorescent Labeled Lectin to 100 µg/ml using Buffer.</li> </ol>
<ul> <li>Ref. M. Immbar et. al., (1973). Intnl. Journal of Cancer, 12, 93-99</li> <li>Cell Suspension</li> <li>1. Wash cells with Buffer (See reverse side.)</li> <li>2. Collect cells by centrifugation.</li> <li>3. Dilute Fluorescent Labeled Lectin to 100 µg/ml using Buffer.</li> </ul>
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.
<ol> <li>Wash cells with Buffer (See reverse side.)</li> <li>Collect cells by centrifugation.</li> <li>Dilute Fluorescent Labeled Lectin to 100 µg/ml using Buffer.</li> </ol>
<ol> <li>Collect cells by centrifugation.</li> <li>Dilute Fluorescent Labeled Lectin to 100 µg/ml using Buffer.</li> </ol>
3. Dilute Fluorescent Labeled Lectin to 100 µg/ml using Buffer.
<ol> <li>Include approximately 1x10 cells with 1 mi diluted Fluorescent labeled Lectin for 15 minutes at room temperature or in a 37°C water bath.</li> </ol>
<ol><li>Wash cells with Buffer three times using centrifugation.</li></ol>
<ol><li>Examine cells, with or without fixation with Fluorescent microscope. Use appropriate filter.</li></ol>
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 <sup>TM</sup> 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 <b>Fluorescent Labeled Lectin</b> with inhibitory carbohydrate for 30-60 minutes at
room temperature before applying to section or cells.
TROUBLE SHOOTING GUIDE
Problem Cause Solution
1. Low concentration of specific Causes #1 - #3
Weak or no oligosaccharide on sample. a. Increase incubation time.
2. Low concentration of lectin conjugate. b. Increase concentration conjugate.
3. Insufficient incubation time.
4. Photobleaching     a. Avoid exposure to light.       1. Lectin conjugate is too concentrated.     a. Decrease concentration of Lectin conjugate.
a. Decrease concentration of Lectin conjugate.
2. Insufficient washing. a. Perform multiple washings and prolong
High washing time.
Background 3. Autofluorescent sample. a. Use fluorochrome with different excitation
and emission spectrum.
b. Use a different lectin conjugate (enzyme <u>or</u>
colloidal gold).
Unexpected Multiple senses a. Perform control reactions.
Staining Pattern Multiple causes b. Use other cytochemical technique to prove or disprove the findings.
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#### MSDS for Fluorescent labeled Purified Proteins Continued - page 2 of 2.

#### **MATERIAL SAFETY DATA SHEET**

Effective Date: March 31, 2006 Revision 4 Page 1 of 2

#### **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	FP-01, RP-01, TP-01, F-1102 to F-9000, R-1102 to R-9000, T-1102 to T-9000, FA-
Number (s):	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 $\operatorname{Red}^{\otimes}$

#### **EMERGENCY INFORMATION**

EY Laboratories, Inc. 107 North Amphlett Blvd. San Mateo, CA 94401 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. 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

been thoroughly investigated. Care should be taken when handling any	
	hen handling any of
these materials.	
EFFECTS OF Causes localized eye, skin, or mucous membrane irritation. Some sensiti	tion. Some sensitive
OVEREXPOSURE: individuals may develop a chronic allergic reaction with exposure. T	*
known effects are due to the protein. No specific effects of the bound dye a	of the bound dye are
known at this time.	
ROUTES OF EXPOSURE: Inhalation of powders and skin contact with liquids are the primary routes exposure. Care should be taken to avoid the formation of aerosols where the advices are the power of the advices are the power of t	
handling any of the solutions.	

#### PHYSICAL CHARACTERISTICS

APPEARANCE: SOLUBILITY:

Powders are a light orange. Solutions will be yellow to dark purple. Powders are completely soluble in many biological buffers and water. I liquids are completely miscible in water and biological buffers.

#### FIRE AND EXPLOSION HAZARDS

Not considered to be a vire hazard. At high concentrations the chemicals may emit toxic fumes. Such high concentrations are not normally found in a research laboratory.

EXTINGUISHING MEDIA: SPECTUL FIRE FIGHTING CRECTULIONS:

Dry chemical powder or CO<sub>2</sub>. Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.

# **Y** LABORATORIES, INC.

North Amphlett Blvd. San Mateo, CA 94401 Tel: 650-342-3296 Fax: 650-342-2648 Orders: 1-800-821-0044 (Outside CA only) 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: HAZARDOUS POLYMERIZATION: INCOMPATIBILITY:		Stable. Decomposition products are not known to be hazardous. Will NOT occur. Alcohols, strong bases and acids, strong oxidizing agents, and heat. (Lead and copper may react with sodium azide).		
SPILL / LEAK PROCEDURES				
MATERIAL RELEASE / SPILL:	soaked in hou	ith powder or liquid. Clean up spill with a paper towel sehold bleach. Do not allow solutions to dry on urfaces. Wash affected area with detergent after the area		
WASTE DISPOSAL:	has been treated with bleach. Incinerate, autoclave, or dispose of paper waste in accordance with all Local, State, and Federal regulations. Due to the small quantities of			
		ed these products are generally not considered to be azards. 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	Recommended as a safety precaution, specifically when working with
PROTECTION:	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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