# **PRODUCT INFORMATION FITC Labeled Lectin**

October Neuroberg	E0001.1	your convenience	your convenience. The success of your experiments are not guaranteed by EY Laboratories, Inc.			
Catalog Number:	F-9001-1		Tissue Sections			
Description:	Pure Marasmium oreades agglutinin (MOA) from mushroom, FITC conjugated.	to high leve	<ol> <li>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).</li> </ol>			
			rescent Labeled Lectin to desired concentrati	on 20-100 µg/ml using Buffer.		
Lot Number:			sue section with Fluorescent Labeled Lectin fo	r 30 minutes in a moist chamber.		
			e section with Buffer three times.			
			sue section with Fluorescent microscope. Use			
Protein Concentration: (Based on OD 280)	1 mg purified MOA FITC / 1 ml Buffer.	Ref. M. Im	nbar et. al., (1973). Intnl. Journal of Cancer, 12			
(Dased on OD 200)			Cell Suspensio	n		
FITC / Protein Ratio:			with Buffer (See reverse side.)			
(OD 495/ OD 280)		2. Collect cell	s by centrifugation.			
			rescent Labeled Lectin to 100 µg/ml using Bu			
Purification Procedure:	: Gel filtration performed after conjugation to remove free FITC.	4. Incubate ap temperature	proximately 1x10° cells with 1 ml diluted Fluc or in a 37°C water bath.	prescent labeled Lectin for 15 minutes at room		
			with Buffer three times using centrifugation.			
Carbohydrate Specificity:	Galα 1,3Gal and Galα1,3 Galβ 1,4GlcNAc.		lls, with or without fixation with Fluorescent m	icroscope. Use appropriate filter.		
Specificity.			ss. (1977). Experimental Pathology, 14, S15			
Inhibitory Carbohydrate:	Galα 1,3Gal and Galα1,3 Galβ1,4GlcNAc.	Fluorochromes r covered in foil.	Fluorochromes must be protected from light. Perform incubation, when practical, in a dark room or covered in foil.			
			Absorption and Em	ission		
Activity:	N/A		Absorption/Excitation Ra	te Emission Max.		
			TC 492 nm	517 nm		
Buffer:	0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4. Contains 0.05% sodium azide as a		RITC 554 nm	570 nm 615 nm		
Bullel.	preservative.	Texas Red™ 596 nm 615 Carbohydrate Inhibition				
Chamical Lload for		Inhibition of lectu	binding may be accomplished by using one of			
Chemical Used for Conjugation:	Fluorescein Isothiocyanate, FITC.			, incubate section or cells with inhibitory		
eenjaganeni		carbohydra	te for 30-60 minutes at room temperature. NO	TE: Complete inhibition may NOT occur.		
Storage:	Store refrigerated at 5-8°C or frozen. For long term storage, store liquid frozen in	B. Preincubate	diluted Fluorescent Labeled Lectin with	inhibitory carbohydrate for 30-60 minutes at		
-	aliquots. Avoid freeze-thaw cycles. Clarify by centrifugation.	room tempe	erature before applying to section or cells.			
			TROUBLE SHOOTING GUIDE			
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	<b>D</b> 11				
	0.05% soutuin azue audeu as a preservative	Problem	Cause           1. Low concentration of specific	Solution Causes #1 - #3		
Caution:	Refer to the enclosed MSDS for information regarding lectins. The aluminum		oligosaccharide on sample.	a. Increase incubation time.		
	seals have sharp edges and the vial itself may have cracks which can cause lacera-	Weak or no Staining	2. Low concentration of lectin conjugate.	b. Increase concentration conjugate.		
	tions. Use caution when opening the vial.	Stalling	3. Insufficient incubation time.			
<b>_</b>			<ol> <li>Photobleaching</li> <li>Lectin conjugate is too concentrated.</li> </ol>	<ul><li>a. Avoid exposure to light.</li><li>a. Decrease concentration of Lectin conjugation</li></ul>		
Remarks:	Puorescent Conjugates are extremely light sensitive.		1. Lecuir conjugate is too concentrated.	<ul> <li>b. Shorten incubation times.</li> </ul>		
			2. Insufficient washing.	a. Perform multiple washings and prolong		
References:	Winter, H.C., et al. (2002) Biochem.J. 277:14996-15001.	High		washing time.		
	<ol> <li>Kruger, R.P., et al. (2002) J. Biochem. 277:15002-15005.</li> <li>Trachara S. et al. (2002) Charachichem 12 470-486</li> </ol>	Background	3. Autofluorescent sample.	<ul> <li>a. Use fluorochrome with different excitatio and emission spectrum.</li> </ul>		
(( )) *	3. Teneberg, S., et al. (2003) Glycobiology. <b>13</b> :479-486.			b. Use a different lectin conjugate (enzyme		
$\checkmark$				colloidal gold).		
				a. Perform control reactions.		
$ \square $		Unexpected				
		Unexpected Staining Pattern	Multiple causes	b. Use other cytochemical technique to prov		
NO -			Multiple causes			
			Multiple causes	b. Use other cytochemical technique to prov		

# **General Procedure** Fluorescent Labeled Lectin

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

## **Tissue Sections**

	bing levers of non-specific dackground. After blocking, finse briefly wind buffer (see reverse side).				
	Dilute Fluorescent Labeled Lectin to desired concentration 20-100 µg/ml using Buffer.				
	Incubate tissue section with Fluorescent Labeled Lectin for 30 minutes in a moist chamber.				
	Wash tissue section with Buffer three times.				
		ction with Fluorescent microscope. Use			
Ref. N	1. Immbar et	. al., (1973). Intnl. Journal of Cancer, 12	2, 93-99		
		Cell Suspensio	n		
1. Wash	cells with Bu	Iffer (See reverse side.)			
2. Collec	t cells by cer	ntrifugation.			
	-	t Labeled Lectin to 100 µg/ml using Bu	uffer.		
	Incubate approximately 1x10 <sup>6</sup> cells with 1 ml diluted Fluorescent labeled Lectin for 15 minutes at room temperature or in a $37^{\circ}$ C water bath.				
5. Wash	cells with Bu	Iffer three times using centrifugation.			
6. Exam	ine cells, with	h or without fixation with Fluorescent m	icroscope. Use appropriate filter.		
Ref. K	. Phiss. (197	7). Experimental Pathology, 14, S15			
	mes must be		pation, when practical, in a dark room or		
		Absorption and Em	ission		
		Absorption/Excitation Ra			
	FITC TRITC	492 nm	517 nm		
	Texas Re	554 nm 596 nm	570 nm 615 nm		
	Texas Re	596 IIII	615 lilli		
		Carbohydrate Inhil	pition		
Inhibition of	lectin bindir	ng may be accomplished by using one of	two procedures:		
A. Before	e incubating	g with Fluorescent Labeled Lectin	, incubate section or cells with inhibitory		
carbol	hydrate for 3	0-60 minutes at room temperature. NO	TE: Complete inhibition may NOT occur.		
			inhibitory carbohydrate for 30-60 minutes at		
room	temperature	before applying to section or cells.			
		TROUBLE SHOOTING	GUIDE		
Problem		Cause	Solution		
		Low concentration of specific	Causes #1 - #3		
Weak or		oligosaccharide on sample.	a. Increase incubation time.		
Stainin		Low concentration of lectin conjugate. Insufficient incubation time.	b. Increase concentration conjugate.		
		Photobleaching	a. Avoid exposure to light.		
		Lectin conjugate is too concentrated.	a. Decrease concentration of Lectin conjugate.		
		zeenn eonjugate is too eoneennaatea.	b. Shorten incubation times.		
	2. 1	Insufficient washing.	a. Perform multiple washings and prolong		
High Background		0	washing time.		
	and 3. A	Autofluorescent sample.	a. Use fluorochrome with different excitation		
			and emission spectrum.		
			b. Use a different lectin conjugate (enzyme <u>or</u>		
1			colloidal gold).		
			a Derform control reactions		
Unexpec	Miii	ltiple causes	a. Perform control reactions.		
Unexpec Staining Pa	Miii	ltiple causes	<ul><li>a. Perform control reactions.</li><li>b. Use other cytochemical technique to prove or disprove the findings.</li></ul>		



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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**

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
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.
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

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	Causes localized eye, skin, or mucous membrane irritation. Some sensitive
OVEREXPOSURE:	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: 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: SPECIAL FIRE FIGHTING CRECAUTIONS:

Dry chemical powder or CO<sub>2</sub>. 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: 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 hous	ith powder or liquid. Clean up spill with a paper towel ehold bleach. Do not allow solutions to dry on rfaces. 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			
	material involved	d Federal regulations. Due to the small quantities of d these products are generally not considered to be zards. 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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