DATA SHEET

PRODUCT INFORMATION
FITC Labeled Lectin

Catalog Number: F-3901-2

Description: Pure *Maclura pomifera* lectin (MPA) from Osage Orange, FITC conjugated.

Lot Number:

Protein Concentration: (Based on OD 280) 2 mg purified MPA FITC /2 ml Buffer.

FITC / Protein Ratio: (OD 495/ OD 280)

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

Carbohydrate Specificity: N-Acetylgalactosamine>Galactose.

Inhibitory Carbohydrate: Melibiose [Gal α(1,6) Glc]>α-D-Galactose.

Activity: Less than 5 µg/ml will agglutinate type O human erythrocytes. Less than 0.1 µg/ml will agglutinate neuraminidase treated cells.

Buffer: 0.02 M Sodium Bicarbonate, pH 9.0-9.5. Contains 0.05% sodium azide as a preservative.

Chemical Used for Conjugation: Fluorescein Isothiocyanate, FITC.

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 lacera- tions. Use caution when opening the vial.

Remarks: Fluorescent Conjugates are extremely light sensitive.

References:

1. Bausch, N.J,. et.al. (1977) Biochem. **16**:5790.
2. Jones, J.M., et.al. J.D. (1973) J. Immunol. **111**:1765.
3. Bird, G.W.G., et.al. (1973) Vox Sang, **24**:48.

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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