

## **FITC labeled Lectin**

## **Product Description**

Name :		Pure Griffonia simplicifolia lectin (GS-I), FITC conjugated	
Catalog Number :		FP-MS9020 2 mg purified GS-I FITC / 2 ml Buffer	
Absorption / Emission :		$\lambda_{\rm exc} \lambda_{\rm em} = 492 / 517 \text{ nm}$	
<b>Purification Procedure :</b>		Gel filtration performed after conjugation to remove free FITC	
Carbohydrate Specificity :		Melibiose, α-D-Galactose	
Inhibitory Carbohydrate :		α-Galactose	
Activity :		20-30 µg/ml is required to agglutinate fresh type B blood cells. Lectin activity	
Buffer :		against all blood types increases after neuraminidase treatment of the cells. 0.01M Phosphate - 0.15M NaCl containing 0.5 mM CaCl2, pH 7.2 - 7.4. Contains 0.05% sodiumazide as a preservative.	
		contains 0.0570 sourainazide as a preservative.	
Storage:	Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze thaw cycles. Clarify by centrifugation. Protect from light and moisture.		
Stability:	The liquid material is stable for at least 1 year when stored frozen in aliquots with 0.05% sodiu azide added as a preservative.		

## **Directions for use**

#### Remarks

Calcium is REQUIRED for binding. 0.5mM Calcium is the maximum concentration in Buffer that will not form a white precipitate.

Fluorescent Conjugates are extremely light sensitive.

#### Procedure

The following is a general Procedure and Trouble-Shooting Guide. The information is provided only for your convenience.

#### **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  $100 \ \mu g/ml$  using Buffer.

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#### FT-MS9020

- 4. Incubate approximately 1x106 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.

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

#### References

- Goldstein I.J. et al., Adv. Carbohydrate Chem. 35, 127 (1978)
- Judd W.J. et al, Vox Sang, 33, 246 (1977)
- Shankar Iyer, P.N., et al, Arch. Biochem. Biophys., 177, 330 (1976)

## **Technical and scientific information**

#### Related / associated products and documents

See BioSciences Innovations catalogue and e-search tool.

• ConA-FITC, <u>FP-47496A</u>

• Other <u>conjugated lectins</u>

## **Ordering information**

<u>Catalog size quantities and prices may be found at www.interchim.com/</u> Please inquire for higher quantities (availability, shipment conditions).

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For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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