FT-FO0061

FITC labeled Lectin Staining Kit #1

Product Description

The FITC Labeled Lectin Staining Kit #1 contains 1mg each of the labeled lectins:
Con A, DBA, SBA,WGA, UEA-I, PNA, GS-I, GS-II, BPA, MPA.

Lectin Specificity

Con A	α-D-Mannose, α-D-Glucose, Branched mannose.
DBA	Methyl-2-acetamido-2-deoxy-D-galactose.
SBA	α and β- N-Acetylgalactosamine > α and β-Galactose.
WGA	(GlcNAc-β-(1,4)-GlcNAc)1 -4 > β-GlcNAc > Neu5Ac.
UEA-I	α-L-Fucose.
PNA	Terminal β-Galactose.
GS-I	Melibiose, α-D-Galactose.
GS-II	Terminal α or β- N-Acetylglucosamine. The specific linkage of the N-Acetylglucosamine to the subterminal carbohydrate plays an important role in lectin binding
BPA	N-Acetylgalactosamine.
MPA	N-Acetylgalactosamine > Galactose.

Name :	**Pure *Canavalia ensiformis* lectin (Concanavalin A, Con-A) from Jackbean, FITC conjugated**
Catalog Number :	FP-MS8931 1 mg
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ = 495 / 517 nm
Presentation:	0.05 M Tris - 0.15M NaCl-0.004M CaCl2, pH 7.0-7.2. Contains 0.05% sodium azide as a preservative
Carbohydrate Specificity :	α-D-Mannose, α-D-Glucose, Branched mannose
Inhibitory Carbohydrate :	Methyl α-D-Mannopyranoside >> α-D-Mannose>> α-D-Glucose.
Activity :	Con A is a relatively weak blood agglutinin More than 10 µg/ml may be required to give visible agglutination of neuraminidase treated human erythrocytes.

Name :	**Pure *Triticum vulgare* lectin (WGA) from Wheat Germ Agglutinin, FITC conjugated**
Catalog Number :	FP-CE8071 1mg /1 ml buffer
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ = 495 / 517 nm FITC / Protein Ratio: (OD 495 / OD 280)
Carbohydrate Specificity:	$(GlcNAc\text{-}\beta\text{-}(1,4)\text{-}GlcNAc)_{1\text{-}4}$>β-GlcNAc>Neu5Ac.
Inhibitor Carbohydrate:	GlcNAc β(1,4) GlcNAc β(1,4) GlcNAc>GlcNAc β(1,4) GlcNAc> GlcNAc>>sialic acid(Neu5Ac)>>GalNAc.
Activity:	Less than 4mg/ml will agglutinate human type O erythrocytes. Less than 1 µg/ml will agglutinate neuraminidase treated erythrocytes.
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.
References:	1. Nagata, Y., et.al. (1974) J.Biol.Chem. 249:3316. 2. Goldstein, I.J., et al., (1975) Biochem.Biophys.Acta. 405:53. 3. Rice, R.H. ,et.al. (1975) Biochem. 14:4093.

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Name :	**Pure *Griffonia simplicifolia* lectin (GS-I), FITC conjugated**
Catalog Number :	FP-MS9021 1 mg purified GS-I FITC / 1 ml Buffer.
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ = 495 / 517 nm
Buffer:	0.01M Phosphate - 0.15M NaCl containing 0.5 mM CaCl2, pH 7.2 – 7.4. Contains0.05% sodiumazide as a preservative.
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 against all blood types increases after neuraminidase treatment of the cells.
Remarks :	Calcium is REQUIRED for binding. 0.5mM Calcium is the maximum concentration in Buffer that will not form a white precipitate.

Name :	**Pure *Griffonia simplicifolia* lectin (GS-II), FITC conjugated**
Catalog Number :	FP-MS9031 1 mg purified GS-II FITC / 1 ml Buffer.
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ = 495 / 517 nm
Buffer:	0.01M Phosphate - 0.15M NaCl containing 0.5 mM CaCl2, pH 7.2 – 7.4. Contains0.05% sodiumazide as a preservative.
Carbohydrate Specificity :	Terminal α-or β-N-Acetylglucosamine. The specific linkage of the N-Acetylglucosamine to the subterminal carbohydrate plays an important role in lectin binding.
Inhibitory Carbohydrate :	N-Acetylglucosamine.
Activity :	5-10 µg/ml will agglutinate T_k polyagglutinable cells.
Remarks :	Calcium is REQUIRED for binding. 0.5mM Calcium is the maximum concentration in Buffer that will not form a white precipitate.

Name :	**Pure *Bauhinia purpurea* lectin (BPA) from Camel's foot tree, FITC conjugated**
Catalog Number :	FP-MS9041 1 mg purified BPA FITC / 1 ml Buffer.
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ = 495 / 517 nm
Buffer:	0.01M Phosphate - 0.15M NaCl containing 0.5 mM CaCl2, pH 7.2 – 7.4. Contains0.05% sodiumazide as a preservative.
Carbohydrate Specificity :	N-Acetylgalactosamine.
Inhibitory Carbohydrate :	N-Acetylglucosamine.
Activity :	Less than 0.5 µg/ml will agglutinate human erythrocytes after neuraminidase treatment of the cells. Without prior enzyme treatment, at least 25 µg/ml is required to agglutinate red blood cells.

Name :	**Pure *Maclura pomifera* lectin (MPA) from Osage Orange, FITC conjugated**
Catalog Number :	FP-MT0220 1 mg purified MPA FITC / 1 ml Buffer.
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ = 495 / 517 nm
Buffer:	0.02M Sodium Bicarbonate, pH 9.0-9.5. Contains 0.05% sodium azide as a preservative.
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.

Name :	**Pure *Arachis hypogaea* lectin (PNA) from peanut, FITC conjugated**
Catalog Number :	FP-MT0220 1 mg purified PNA FITC / 1 ml Buffer.
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ = 495 / 517 nm
Buffer:	0.02M Sodium Bicarbonate, pH 9.0-9.5. Contains 0.05% sodium azide as a preservative.
Carbohydrate Specificity :	Terminal β-Galactose
Inhibitory Carbohydrate :	Lactose > β-Galactose
Activity :	Less than 1 µg/ml will agglutinate human erythrocytes neuraminidase treatment of the cells.

Name :	**Pure *Glycine max* lectin (SBA) from Soybean, FITC conjugated**
Catalog Number :	FP-MS6140 1 mg purified SBA FITC / 1 ml Buffer.
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ = 495 / 517 nm
Buffer:	0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4. Contains 0.05% sodium azide as a preservative.
Carbohydrate Specificity :	α and β-N-Acetylgalactosamine > α and β-Galactose
Inhibitory Carbohydrate :	Terminal α– and β-N-Acetylgalactosamine > Galactose
Activity :	Less than 4 µg/ml will agglutinate fresh A1 cells. Older B cells can react stronger than A2 cells.

Name :	**Pure *Sambucus nigra* lectin (SNA-I), from elderberry - FITC conjugated**
Catalog Number :	FP-MS6340 1 mg purified SNA-I FITC / 1 ml Buffer
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ = 492 /517 nm
Purification Procedure :	Gel filtration performed after conjugation to remove free FITC
Carbohydrate Specificity :	NANA(Neu5Acα(2,6)Gal/GalNAc)
Inhibitory Carbohydrate :	High concentration Lactose > β-Galactose
Activity :	SNA-I agglutinates animal and human erythrocytes. The lectin has a slight preference for type A over B and type O erythrocytes.
Buffer :	0.01M Phosphate - 0.15M NaCl, pH 7.2 – 7.4. Contains 0.05% sodium azide as a preservative.

Name :	**Pure *Ulex europaeus* lectin (UEA-I) from gorse, FITC conjugated**
Catalog Number :	FP-MS0201 1 mg purified UEA-I FITC / 1 ml Buffer.
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ = 495 / 517 nm
Buffer:	0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4. Contains 0.05% sodium azide as a preservative.
Carbohydrate Specificity :	α-L-Fucose
Inhibitory Carbohydrate :	α-L-Fucose
Activity :	Less than 4 µg/ml will agglutinate human type O erythrocytes. Less than 0.5µg/ml will agglutinate neuraminidase treated erythrocytes.
Remarks :	UEA-I contains a high percentage of Ca++ which is required for binding. Treatment of the lectin with EDTA abolishes agglutinating activity. Activity returns with the addition of calcium.

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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% sodium azide added as a preservative.

Directions for use

Remarks

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.
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
2. Collect cells by centrifugation.
3. Dilute **Fluorescent Labeled Lectin** to 100 µg/ml using Buffer.
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 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. Weak or no conjugate. 3. Insufficient incubation time. 4. Photobleaching	Causes #1 - #3 a. Increase incubation time. b. Increase concentration 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.

References

- **Broekaert W.F.** *et al.,* Biochem. J. **221**, 163-169 (1984)
- **Kaifu R.** *et a*l, Carbohydr. Res., **52**, 179-185 (1976)
- **Broekaert W.F**. *et al*, Biochemical Journal, **221**, 163-169 (1984)

Related / associated products and documents

- Lectin List (or search conjugated lectins):
- ConA-Biotin, FP-MS9690; -FITC, FP-47496A; -Cy3, FP-WT8680,
- WGA-biotin, FP-MS5730; -SR101, FP-MS9540; -FITC, FP-CE8070

Ordering information

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

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