FT-MS8930


# FITC - Con A

## Product Description

| | |
|---|---|
| **Name :** | **FITC – Con A** |
| | Pure Canavalia ensiformis lectin (Con A) from Jackbean, FITC conjugated. |
| **Catalog Number:** | FP-MS8930, 2mg |
| **Protein Concentration (based on OD 280):** | 2 mg purified Con A FITC / 2 ml Buffer. |
| **Purification Procedure:** | Gel filtration performed after conjugation to remove free FITC. |
| **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. |
| **Buffer:** | 0.05M Tris – 0.15M NaCl-0.004M $CaCl_2$, pH 7.0-7.2. 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.

## Directions for use

### Guidelines for use

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



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**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 $1x10^6$ 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 |

*Notes:* 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.<br>2. Low concentration of lectin conjugate.<br>3. Insufficient incubation time.<br>4. Photobleaching | Causes #1 - #3<br>a. Increase incubation time.<br>b. Increase concentration conjugate.<br><br>a. Avoid exposure to light. |
| High Background | 1. Lectin conjugate is too concentrated.<br>2. Insufficient washing.<br>3. Autofluorescent sample. | a. Decrease concentration of Lectin conjugate.<br>b. Shorten incubation times.<br>a. Perform multiple washings and prolong washing time.<br>a. Use fluorochrome with different excitation and emission spectrum.<br>b. Use a different lectin conjugate (enzyme or colloidal gold). |
| Unexpected Staining Pattern | Multiple causes | a. Perform control reactions.<br>b. Use other cytochemical technique to prove or disprove the findings. |

## Other technical information

**Concanavalin A (Con A)** is has an affinity for terminal α-D-mannosyl and α-D-glucosyl residues found i.e. in oligosaccharides, glycoproteins and glycolipids. $Ca^{2+}$ and $Mn^{2+}$ ions are required for activity. The pI is 4.5-5.5. Con A dissociates into dimers at pH 5.6 or below. Between pH 5.8 and pH 7.0, Con A exists as a tetramer; above pH 7.0 higher aggregates are formed. Up 4 mannose can bind per ConA, prompting precipitation and agglutination properties. Affinity constants are in the range of $K_d = 10^{-6} - 10^{-7}$ mol/L for glycoproteins.



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ConA lies in its specific binding action with certain carbohydrate-containing receptors. It complexes with blood group substances[r] and immunoglobulin glycopeptides[rr] and carcinoembryonic antigen[r]. It is reported to interact with human plasma low density lipoprotein[r] and with lipopolysaccharide[r].

ConA is then, once coupled to a resin, used for purifications, and once labeled (i.e. by biotin or a a fluorophore) for detection techniques such as blotting, flow cytometry, IHF,... but also cell aglutinations or carbohydrate precipitations, for carbohydrates and cells studies:

Immobilized specific lectins are useful for purifying glycoprotein[r] and removing contaminants[r]. It has been used to stud y nerve glycoproteins[r]

The conA is not blood group specific, but agglutinates well erythrocytes. Cancer cells are readily aggregated by ConA; normal cells are not[r]. Embryonic cells are also aggregatable[r]. Normal cells react after proteolytic treatment (trypsinization), that may cause clustering of the membrane ConA sites[r]. On the other hand, it is reported that ConA treated with trypsin can restore growth patterns of transformed fibroblasts to normal[r].
Many studies adress ConA with particular cell types, including locust muscle fibers[r]; lymphocytes[r]; fibroblasts[r]; adipocytes[r]; rat liver plasma membrane components[r]. ConA induces endo-reduplication in mammalian cells[r] and induces oocyte maturation-inducing substance in starfish follicle cells[r]. ConA reaction is reported with E. coli[r]; that with Dictyostelium discoideum[r]; and that with B. substilis[r].

Con A exhibits mitogenic activity[r] which is dependent on its degree of aggregation. Succinylation results in an active dimeric form which remains a dimer above pH 5.6.

## References


- **Boulan R**. *et al*., Spatial orientation of glycoproteins in membranes of rat liver rough microsomes. I. Localization of lectin-binding sites in microsomal membranes, *J. Cell Biol*., 78: 874 (1978) Article
- **Ito M.** *et al*., Histogenesis of the Intravitreal Membrane and Secondary Vitreous in the Mouse, *Invest. Ophthalmol. Vis. Sci*., 48: 1923 - 1930 (2007) Article
- **Tateno H.** *et al*., Glycoconjugate microarray based on an evanescent-field fluorescence-assisted detection principle for investigation of glycan-binding proteins, *Glycobiology*, 18: 789 - 798 (2008) Article
- Gautier D (fr)) (french)


## Related products

- ConA-FITC, FP-47496A
- ConA-Cy3, FP-WT8680
- ConA-Biotin, FP-MS9690
- GS-I-FITC, FP-MS9020
- PNA-FITC, FP-BV4181
- WGA-biotin, FP-MS5730
- WGA-SR101, FP-MS9540
- WGA-FITC, FP-CE8070
- Other reagents: BSA, UPQ84170
- Streptavidin – FluoProbes 547H, FP-CA5570

## 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


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