FT-MS9020

Biotin – Con A

Product Description

Name :	**biotin – Con A**
	Biotin Conjugated *Canavalia ensiformis* Lectin (Jackbean) - Con A
Catalog Number:	FP-MS9690, 5mg
Protein Concentration (based on OD 280):	5 mg purified Con A Biotin / vial, lyophilized. Reconstitute with distilled water to a final concentration of 1 mg/ml
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.
Chemical Used for Conjugation:	Biotinyl N - hydroxysuccinimide ester (BNOHSE)

Storage: Store lyophilized powder refrigerated at 5 - 8°C or frozen. Store liquid frozen in aliquots. Avoid freeze-thaw cycles
Stability: The lyophilized material is stable for several years when stored frozen. After reconstitution the 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.

1. Wash and block tissue section or blot. It is recommends that 1% purified Bovine Serum Albumin (BSA) or defatted milk powder be used for blocking to prevent nonspecific binding. Do not use serum products, they contain glycoproteins which may lead to high levels of non specific background. After blocking, rinse briefly with recommended Buffer.
2. Dilute **Biotin Labeled Lectin** to a concentration of 5-50 µg/ml using recommended Buffer. Incubate section or blot for 30-90 minutes at room temperature in a moist chamber. Slightly longer incubation times may be required if incubation is done at 2-8ºC. Rinse 3 times, 5 minutes each time with recommended Buffer.
3. Dilute and incubate **Avidin Conjugate** according to manufacturer directions.

Notes: Inhibition of lectin binding may be accomplished by using one of two procedures:
A. Before proceeding to Step #3 incubate lectin treated section or blot with inhibitory carbohydrate for 30-60 minutes at room temperature. NOTE: Complete inhibition may not occur.

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B. Preincubate diluted Biotin Labeled Lectin with inhibitory carbohydrate for 30-60 minutes at room temperature before applying to section or blot.

Problem	Cause	Solution
Weak or no Staining	1. Low concentration of specific oligosaccharide on sample. 2. Low concentration of lectin conjugate. 3. Low concentration of avidin conjugate. 4. Insufficient incubation time. 5. Inappropriate treatment of sample prior to labeling.	Causes #1 - #4 a. Increase incubation time. b. Increase concentration of sample (on blot) lectin conjugate and/or avidin conjugate. a. Treat section or blot with a different blocking reagent.
High Background	1. Lectin conjugate and/or avidin conjugate is too concentrated. 2. Insufficient washing. 3. Insufficient blocking. 4. Sample contains endogenous enzymatic activity.	a. Decrease concentration of respective reagents. b. Shorten incubation times. a. Perform multiple washings and prolong washing time. a. Treat section or blot with a different blocking reagent. a. Determine if sample contains activity which would give background staining in the absence of the avidin conjugate.
Unexpected Staining Pattern	Multiple causes	a. Perform control reactions. 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.

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.

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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 / associated products and documents

See BioSciences Innovations catalogue and e-search tool.

- Lectin List (or search conjugated lectins):
- 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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