FT-MS9960

# Wheat Germ Agglutinin - TRITC

This Lectin recognizes (N-acetyl glucosamine or chitobiose) (structure common to many serum proteins, membrane glycoprotein, chitin, cartilage, glucosaminoglycans, and glycolipids ) and glycoproteins with sialic acid residues.

## Product Information

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|---|---|
| **Name :** | Wheat Germ Agglutinin (WGA) – TRITC |
| **Catalog Number :** | FP-MS9960 5 mg / 5 ml buffer |
| **Purification procedure:** | Gel filtration performed after conjugation to remove free dye |
| **Carbohydrate Specificity:** | $(GlcNAc-\beta-(1,4)-GlcNAc)_{1-4}>\beta$-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. |
| **Buffer:** | 0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4. Contains 0.05% sodium azide as a preservative. |
| **Molecular Weight :** | MW= 36 000 kDa (2 subunits of 18 000 kDa each) |
| **Concentration:** | 1 mg/ml |
| **Absorption / Emission :** | $\lambda_{ex\backslash em}$ = 554 / 570 nm |

**Storage:** Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze thawcycles. Clarify by centrifugation.

The liquid material is stable for at least 1 year when stored frozen in aliquots with 0.05%sodium azide added as a preservative.

## Introduction

The lectin used in this product is a research grade affinity-purified wheat germ agglutinin (WGA).
Main carbohydrate recognition characteristics: β N-acetyl glucosamine-residues and its polymers. Total activity of conjugate against an affinity matrix: >95%.

## Directions for use

### Guidelines for use

Application: Immunofluorescence 10-20 micrograms/ml

Do not dilute the entire reconsituted solution at once. Withdraw aliquots as needed with a micropipette and keep concentrated stock at 4°C. Dilute according to the particular application being used with the provided buffer.

### General Procedure - Fluorescent Labeled Lectin

The following is a general Procedure given for your convenience, that should be optimized and adapted to your technique and conditions. See also below the Trouble-Shooting Guide.

**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 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

**Notes**

Fluorochromes must be protected from light: light exposure durin unproper storage may affect the reagent quality, and should be limited as possible also during operating because Fluorescein bleaches: Perform incubation, when practical, in a dark room or covered in foil. For a photostable fluorescent dye, use biotin labeled WGA and streptavidin-FluoProbes 488.

| Dye | Absorption and Emission | Reading: 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 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 Staining | 1. Low concentration of specific oligosaccharide on sample. | Causes #1 - #3<br>a. Increase incubation time.<br>b. Increase concentration Weak or no conjugate. |

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|---|---|---|
| | 2. Low concentration of lectin conjugate. | |
| | 3. Insufficient incubation time. | |
| | 4. Photobleaching | a. Avoid exposure to light. |
| High Background | 1. Lectin conjugate is too concentrated. | a. Decrease concentration of Lectin conjugate.<br>b. Shorten incubation times. |
| | 2. Insufficient washing. | a. Perform multiple washings and prolong High washing time. |
| | 3. Autofluorescent sample. a. | 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. |

## Related products

- ConA-FITC
- Fluoro-Gel mounting medium, FP-AL2561

### References - WGA

- **Reyes A**.,et al. Chitin synthase III requires Chs4p-dependent translocation of Chs3p into the plasma membrane, *J. Cell Sci*., 120: 1998 - 2009 (2007)
- **Baurand A.** et al., ß-Catenin Downregulation Is Required for Adaptive Cardiac Remodeling, Circ. Res., 100: 1353 - 1362 (2007)

Not dangerous, not regulated product (MSD on inquire).

## Ordering information

Catalog size quantities and prices may be found at http://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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