Wheat Germ Agglutinin (WGA) - AcalephFluor488 User Manual

Catalog # CRG1130

AcalephFluor488 labled Wheat Germ Agglutinin (WGA) can bind N-acetylglucosamine and N-acetylneuraminic acid residues on glycoconjugates and oligosaccharides found throughout the cell surface

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Wheat germ agglutinin (WGA) is a cell impermeant stain that selectively binds to N-acetylglucosamine and N-acetylneuraminic acid (sialic acid) residues, which are often found on cell membranes. Since WGA binds to glycoconjugates itsderivatives and conjugates are widely used to label cell membranesand fibrotic scar tissue for fluorescence imaging and analysis. Thecarbohydrate-binding specificity of WGA is directed against sequencesof β-1,4-GlcNAc-linked residues, the chitodextrins. Each monomercontains two identical, non-interacting binding sites which arecomplementary to 3 or 4 β-1,4-GlcNAc units. Of the monosaccharidesexamined, only GlcNAc binds to WGA. ManNAc does not bind andGalNAc binds only weakly. WGA binds with high affinity to internalGlcNAc residues in large oligosaccharides containing repeat sequencesof Gal beta(1-4)GlcNAc beta(1-3). N-Acetylneuraminic acid is involved only in low-affinityinteractions with WGA. WGA displays an intricate pattern of saccharidespecificities that might be used for structural analysis of complexcarbohydrates.

II. MATERIALS REQUIRED BUT NOT PROVIDED

1. PBS (1X)
2. 4% Formaldehyde
3. 0.5% Triton X-100
4. DAPI
5. Antifade Mounting Medium

III. WORKING SOLUTIONPREPARATION

Stock Solution (200X):Dissolve the lyophilized powder in 300µl$ddH_2O$for the 300 Assays size or 50 µlddH_2O for the 50 Assays size.

Working Solution: Dilute 1 µl fluorescent WGA stock solution in 200 µl PBS before use.

(For fluorescent WGA, the recommended dilution ratio is 1:100-1:200, one time experiment is equivalent to 1-2 µl stock solution in a total staining volume of 200 µl.)

Note: The dilution ratio can be adjusted appropriately according to the experimental effect.

Note:

The reconstituted conjugate solution can be stored at 2-8 °C forshort-term storage or at -20 °C for long-term storage. Avoid repeated freeze-thaw cycles.

IV. ASSAY PROCEDURE

Staining fixed cells (24-well plate/Cell slide)

1. Wash cells 3 times with PBS.

2. Fix cells on ice with 4% formaldehyde solution in PBS for 15 minutes.

Note: For fixed cell membrane staining, it is recommended tostain without the permeabilization step. A permeabilization stepafter fixation can facilitate staining intracellular compartmentssuch as Golgi and Endoplasmic Reticulum (ER) structures.

3. Wash cells 3 times with PBS.

4. Add 200 µL WGA Working Solution,incubate for 10-30 minutes atroom temperature.

5. Wash 2-3 times with PBS.

6. Stain the nucleus (optional): Add DAPI staining solution to cover the cells on the sample, perform cell nucleus staining for 5-10 minutes. Wash the sample 2-3 times with PBS, each time for 5 minutes.

7. Image using fluorescence microscopy. FluorescentWGA are photostable enough to image in PBS, but for best results we recommend mounting with antifade mounting medium.

Staining living cells (24-well plate)

1. Wash cells twice with PBS buffer.

2. Add 200 µL WGA Working Solution.

3. Incubate cells with WGA Working Solution for 10-30 minutes at 37°C.

4. Wash cells twice with PBS buffer.

5.Image using fluorescence microscopy.

Tissue section staining

1. Pre-treatment of tissue sections

Frozen sections were left at room temperature for several minutes to restore to room temperature; the sections were immersed in the fixative at room temperature for 10-15 minutes, then removed and naturally dried, and then rinsed with pure water or PBS to remove the residual fixative on the tissue.

Paraffin sections were placed in the dewaxing solution for 10 minutes, then immersed in anhydrous ethanol for 10 minutes, 95% ethanol for 10 minutes, 80% ethanol for 10 minutes, and distilled water for rinsing.

2. Antigen retrieval, sections were placed in Tris-EDTA pH 8.0, and heated for 15 minutes for retrieval. After natural cooling, the slides were placed in PBS and washed 3 times, each time for 5 minutes.

3. Add 50-100 µl of WGA Working Solution to cover the tissue, incubate at 37°C in the dark for 30 minutes.

4. Wash the sample 3 times with PBS, each time for 5 minutes.

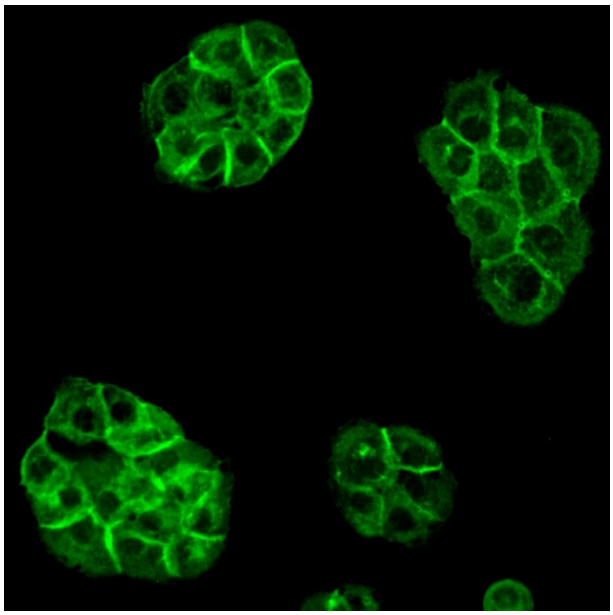
5. Stain the nucleus (optional): Add DAPI staining solution to cover the cells on the sample, perform cell nucleus staining for 5-10 minutes. Wash the sample 2-3 times with PBS, each time for 5 minutes.

6. After slightly drying the section, add Antifade Mounting Medium on the sample and use a suitable cover slip for mounting.

7. Image using fluorescence microscopy.

V. FLUORESCENCE SPECTRUM

Conjugation	Excitation	Emission
Wheat Germ Agglutinin (WGA) - AcalephFluor405	404 nm	431 nm
Wheat Germ Agglutinin (WGA) - AcalephFluor488	491 nm	512 nm
Wheat Germ Agglutinin (WGA) - AcalephFluor532	525 nm	554 nm
Wheat Germ Agglutinin (WGA) - AcalephFluor555	555 nm	565 nm
Wheat Germ Agglutinin (WGA) - AcalephFluor568	575 nm	598 nm
Wheat Germ Agglutinin (WGA) - AcalephFluor594	593 nm	614 nm
Wheat Germ Agglutinin (WGA) - AcalephFluor633	630 nm	650 nm
Wheat Germ Agglutinin (WGA) - AcalephFluor647	648 nm	664 nm
Wheat Germ Agglutinin (WGA) - AcalephFluor660	663 nm	682 nm
Wheat Germ Agglutinin (WGA) - AcalephFluor680	681 nm	698 nm
Wheat Germ Agglutinin (WGA)-AcalephFluor750	750 nm	777 nm



Cells were stained with Wheat Germ Agglutinin (WGA) - AcalephFluor488