AAT Bioquest®

PRODUCT INFORMATION SHEET

iFluor® 532-Wheat Germ Agglutinin (WGA) Conjugate

Catalog number: 25532
Unit size: 1 mg

Component	Storage	Amount (Cat No. 25532)
iFluor® 532-Wheat Germ Agglutinin (WGA) Conjugate	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

Wheat germ agglutinin (WGA) is a well-studied lectin known for its binding affinity to N-acetyl-D-glucosamine and sialic acid, making it a valuable tool in various biological applications. Its interaction with glycoconjugates enables widespread use of WGA derivatives and conjugates for fluorescence imaging and analysis, facilitating the labeling of yeast bud scars, fibrotic scar tissue, and the cell membranes of gram bacteria and mammalian cells. WGA specifically targets sequences of β-1,4-GlcNAc-linked residues known as chitodextrins. Each monomer contains two identical, non-interacting binding sites complementary to 3 or 4 β-1,4-GlcNAc units. Among the monosaccharides tested, only GlcNAc shows strong binding to WGA, while ManNAc demonstrates no binding, and GalNAc exhibits weak binding. The iFluor® 532 conjugate of WGA possesses a bright, blue fluorescence characteristic of the iFluor® 532 dye (Ex/Em = 537/560 nm). Notably, the iFluor® 350 WGA conjugate, like Alexa Fluor® 532 WGA conjugate, retains its ability to bind to sialic acid and N-acetylglucosaminyl residues, enhancing its utility in fluorescence imaging and analysis for various scientific investigations.

KEY PARAMETERS

Fluorescence microscope

Emission	Cy3/TRITC filter set
Excitation	Cy3/TRITC filter set
Recommended plate	Black wall/clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

iFluor® 532-Wheat Germ Agglutinin (WGA) Conjugate stock solution (200X)

1. Add 500 µL of ddH_2O into the powder form to make a 2 mg/mL stock solution.

   Note: The reconstituted conjugate solution can be stored at 2-8 °C for short-term storage or at -20 °C for long-term storage.

PREPARATION OF WORKING SOLUTION

iFluor® 532-Wheat Germ Agglutinin (WGA) Conjugate working solution (1X)

1. Add 5 µL of 200X WGA conjugate solution to 1 mL HHBS Buffer.

   Note: The optimized staining concentration may be different with different cell lines. The recommended starting concentration is 5-10 µg/mL for live cells.

SAMPLE EXPERIMENTAL PROTOCOL

Warm the vial to room temperature centrifuge briefly before opening. Staining protocols vary with applications. Appropriate dilution of conjugates should be determined experimentally.

Live Cells Stain

1. Wash cells twice with a HHBS buffer.
2. Add 100 µL iFluor® 532-WGA working solution.
3. Incubate cells with WGA working solution for 10-30 minutes at 37 °C.
4. Wash cells twice with HHBS buffer.
5. Image cells on a fluorescence microscope using Cy3/TRITC filter set.

Fixed Cells Stain

WGA conjugates can be also used to stain fixed cells.

1. Fix cells with 4% Formaldehyde in PBS.

   Note: For fixed cell membrane staining, it is recommended to stain without the permeabilization step. A permeabilization step after fixation can facilitate staining intracellular compartments such as Golgi and Endoplasmic Reticulum (ER) structures.

2. Add 100 µL iFluor® 532-WGA working solution.
3. Incubate cells with WGA working solution for 10-30 minutes at room temperature.
4. Wash cells twice with HHBS buffer.
5. Image cells on a fluorescence microscope using Cy3/TRITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

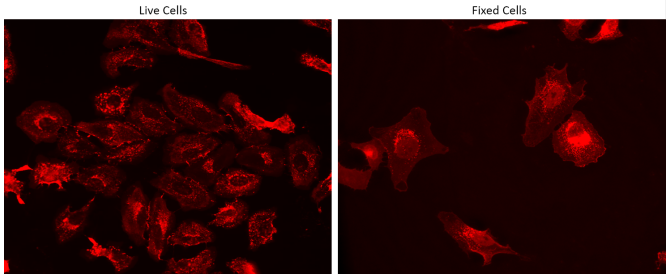

Figure 1. Live and fixed HeLa cells were stained with iFluor® 532-Wheat Germ Agglutinin (WGA) Conjugate at 10 µg/mL for 30 minutes. The image was acquired on a fluorescence microscope using Cy3/TRITC filter set.

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