iFluor® 647-Wheat Germ Agglutinin (WGA) Conjugate

Catalog number: 25559
Unit size: 1 mg

Component	Storage	Amount (Cat No. 25559)
iFluor® 647-Wheat Germ Agglutinin (WGA) Conjugate	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

Wheat germ agglutinin (WGA) is a lectin that binds to N-acetyl-D-glucosamine and sialic acid. It is of the most studied and useful lectins for it biological applications. Since WGA binds to glycoconjugates its derivatives and conjugates are widely used to label cell membranes and fibrotic scar tissue for fluorescence imaging and analysis. The carbohydrate-binding specificity of WGA is directed against sequences of β-1,4-GlcNAc-linked residues, the chitodextrins. Each monomer contains two identical, non-interacting binding sites which are complementary to 3 or 4 β-1,4-GlcNAc units. Of the monosaccharides examined, only GlcNAc binds to WGA. ManNAc does not bind and GalNAc binds only weakly. iFluor®647 conjugate of WGA exhibits the bright, red fluorescence of the iFluor® 647 dye. iFluor®647 WGA conjugate binds to sialic acid and N-acetylglucosaminyl residues as AF647 WGA conjugate does.

KEY PARAMETERS

Fluorescence microscope

Emission	Cy5 filter set
Excitation	Cy5 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® 647-Wheat Germ Agglutinin (WGA) Conjugate stock solution (200X)

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

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® 647-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 Cy5 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® 647-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 Cy5 filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

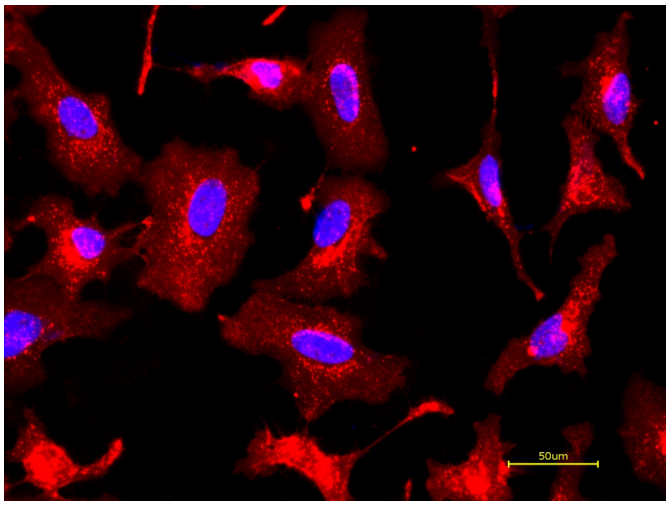

Figure 1. Live HeLa cells were stained with iFluor® 647-Wheat Germ Agglutinin (WGA) Conjugate at 5 µg/mL for 30 minutes followed by Hoechst 33342 (AAT Cat# 17535). Image was acquired using fluorescence microscopy using Cy5 and DAPI filter set.

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