Concanavalin A, AF488 Labeled

Catalog number: 25570
Unit size: 1 mg

Component	Storage	Amount
Concanavalin A, AF488 Labeled	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

Concanavalin A (ConA) is a lectin that binds specifically to certain structures found in various sugars, glycoproteins and glycolipids. ConA is widely used in biology and biochemistry to characterize glycoproteins and other sugar-containing entities on the surface of various cells. It is also used to purify glycosylated macromolecules in lectin affinity chromatography, as well as to study immune regulation by various immune cells. ConA binds specifically α-D-mannosyl and α-D-glucosyl residues (two hexoses differing only in the alcohol on carbon 2) in terminal position of ramified structures from B-Glycans. It has 4 binding sites, corresponding to the 4 subunits. Concanavalin A (Con A) is one of the most widely used lectins in cell biology. AF488-labeled Concanavalin A (equivalent to Alexa Fluor® 488 conjugate of Con A, Alexa Fluor® is the trademark of ThermoFisher) exhibits the bright, green fluorescence of the Alexa Fluor® 488 dye (Ex/Em maxima ~495/519 nm). Alexa Fluor® 488 Con A selectively binds to a-mannopyranosyl and a-glucopyranosyl residues.

KEY PARAMETERS

Fluorescence microscope

Excitation	FITC filter set
Emission	FITC 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.

AF488-Concanavalin A stock solution (200X)
Add 500 µL of ddH_2O into the powder form to make 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

AF488-Concanavalin A Conjugate working solution (1X)
Add 5 µL of 200X 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 AF488-Concanavalin A 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 FITC 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 permeabilization step. Permeabilized step can after fixation will lead to intracellular compartments stain such as Golgi and Endoplasmic Reticulum (ER) structures.
2. Add 100 µL AF488-WGA working solution.
3. Incubate cells with Concanavalin A conjugate working solution for 10-30 minutes at room temperature.
4. Wash cells twice with HHBS buffer.
5. Image cells on a fluorescence microscope using FITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

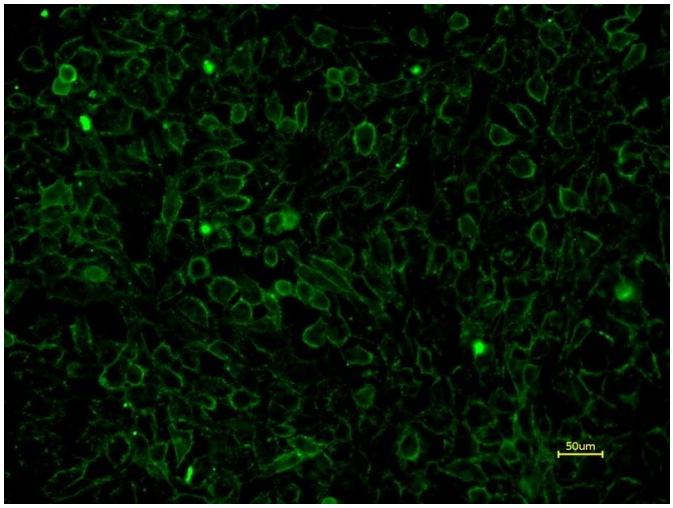

Figure 1. Fluorescence images of Live HeLa cells stained with Concanavalin A, AF488 Conjugate using fluorescence microscope with a FITC filter set (Green).

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