

## iFluor™ 555-Concanavalin A Conjugate

 Catalog number: 25585  
 Unit size: 1 mg

Component	Storage	Amount
iFluor™ 555-Concanavalin A Conjugate	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. Con A is a well-known T cell mitogen that can activate the immune system, recruit lymphocytes and elicit cytokine production. In addition to its mitogenic activity, ConA can induce programmed cell death via mitochondria-mediated apoptosis and autophagy. ConA has also been reported to activate NFAT (nuclear factor of activated T cells), a family of transcription factors that are important in the development and function of the immune system, including T cell receptor (TCR) engagement. 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  $\alpha$ -D-mannosyl and  $\alpha$ -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. iFluor™555-labeled Concanavalin A selectively binds to  $\alpha$ -mannopyranosyl and  $\alpha$ -glucopyranosyl residues and exhibit the bright red fluorescence.

### KEY PARAMETERS

#### Fluorescence microscope

Excitation	Cy3/TRITC filter set
Emission	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™ 555-Concanavalin A Conjugate stock solution (200X)

Add 500  $\mu$ L of ddH<sub>2</sub>O 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

#### iFluor™ 555-Concanavalin A Conjugate working solution (1X)

Add 5  $\mu$ L 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  $\mu$ g/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  $\mu$ L iFluor™ 555-Concanavalin A working solution.
3. Incubate cells with Concanavalin A conjugate 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

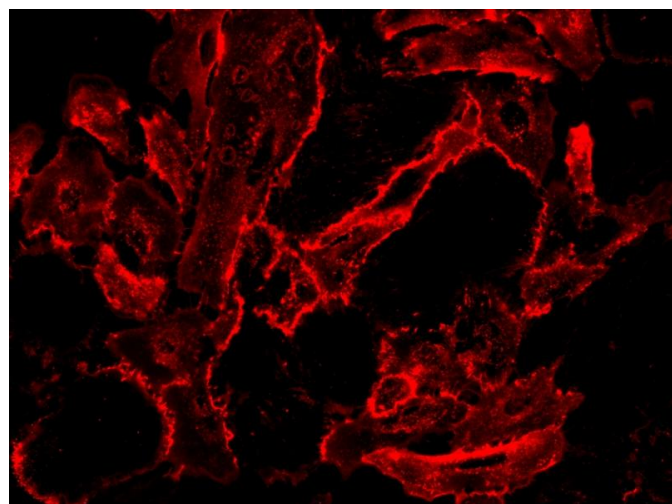
Concanavalin A conjugates can be also used to stain fixed cells.

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  $\mu$ L iFluor™ 555-Concanavalin A working solution.
3. Incubate cells with Concanavalin A 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 HeLa cells were stained with iFluor™ 555-Concanavalin A Conjugate Conjugate at 5  $\mu$ g/mL for 30 minutes. Image was acquired using fluorescence microscopy using Cy3 filter set.

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