# Concanavalin A (ConA)

# -AcalephFluor647

# User Manual

**Catalog # CRG1147**

AcalephFluor647 labled Concanavalin A (ConA) can bind

alpha-D-mannosyl and alpha-D-glucosyl residues from B-Glycans

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

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## I. INTRODUCTION

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. 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.

## 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 SOLUTION PREPARATION

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

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

(For fluorescent ConA, 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 for short-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 to stain without the permeabilization step. A permeabilization step after fixation can facilitate staining intracellular compartments such as Golgi and Endoplasmic Reticulum (ER) structures.

3. Wash cells 3 times with PBS.
4. Add 200 µL ConA Working Solution, incubate for 10-30 minutes at room 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. Fluorescent ConA 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 ConA Working Solution.
3. Incubate cells with ConA Working Solution for 10-30 minutes at 37°C.
4. Wash cells twice with PBS buffer.
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. 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 ConA 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 |
|---|---|---|
| Concanavalin A (ConA) - AcalephFluor405 | 404 nm | 431 nm |
| Concanavalin A (ConA) - AcalephFluor488 | 491 nm | 512 nm |
| Concanavalin A (ConA) - AcalephFluor532 | 525 nm | 554 nm |
| Concanavalin A (ConA) - AcalephFluor555 | 555 nm | 565 nm |
| Concanavalin A (ConA) - AcalephFluor568 | 575 nm | 598 nm |
| Concanavalin A (ConA) - AcalephFluor594 | 593 nm | 614 nm |
| Concanavalin A (ConA) - AcalephFluor633 | 630 nm | 650 nm |
| Concanavalin A (ConA) - AcalephFluor647 | 648 nm | 664 nm |
| Concanavalin A (ConA) - AcalephFluor660 | 663 nm | 682 nm |
| Concanavalin A (ConA) - AcalephFluor680 | 681 nm | 698 nm |
| Concanavalin A (ConA) - AcalephFluor750 | 750 nm | 777 nm |