

## Concanavalin A (with A)-HyperFluor™ 594

### Introduction

Concanavalin A (Con A), derived from *Canavalia ensiformis* (Concanavalin), is a plant lectin protein (Mw = 104 kDa) that binds one Ca<sup>2+</sup> and one Mn<sup>2+</sup> per subunit with a sugar-binding site, and when the metal ions bind to Con A, it can bind various glycoproteins, glycolipids, and α-D-glucose and α-D-mannose moieties in sugars, and HyperFluor™ 594 is a fluorescent dye that fluoresces green (Ex/Em: 590 nm/617 nm) under ultraviolet light. This combination results in a high visualization of concomitant molecules on the cell surface with Con A-HyperFluor™ 594. Concanavalin A-HyperFluor™ 594 is a fluorescently labeled protein that combines Concanavalin A and HyperFluor™ 594 and is widely used in biological research, especially in the field of flow cytometry.

This product is HyperFluor™ 594 conjugated concanavalin A, which is in solution form, which can be used in immunofluorescence and glycobiology research, such as as being used as a probe to detect carbohydrate molecules on the cell surface, because of its ability to bind to a variety of sugars, it can bind to the cell surface expressing a variety of sugars, and can be detected and analyzed by flow cytometry. It is used for immunohistochemical staining to observe the distribution of sugars in tissues or cells by fluorescence microscopy.

### Protocol

#### 1. Working fluid preparation

Take an appropriate amount of product and dilute it with sterile water as a working solution, such as diluting 500 times after taking the product, and prepare 0.01 mg/mL working solution. The recommended working concentration is 5-25 µg/mL, and the specific concentration is recommended to be adjusted according to the experimental needs.

#### 2. Cell staining (The recommended cell concentration is 5-10 µg/mL)

##### 2.1 Staining of live cells

- Wash the cells twice with PBS buffer.
- Add 100 µL of the prepared working solution and incubate the cells at 37°C for 10-30 minutes.
- Wash the cells twice with PBS buffer.
- Cells are imaged on a fluorescence microscope using the Cy3/TRITC filter set.

##### 2.2 Staining of fixed cells

- Fix cells with 4% formaldehyde in PBS. Note: For fixed cell membrane staining, it is recommended to perform staining without a permeabilization step. Permeabilization steps after fixation result in staining of intracellular compartments, such as the Golgi apparatus and endoplasmic reticulum (ER) structures.
- Add 100 µL of the prepared working solution. Incubate for 10-30 min at room temperature.

- c. Wash the cells twice with PBS buffer.
- d. Cells are imaged on a fluorescence microscope using the Cy3/TRITC filter set.

## Note

1. Fluorescent dyes have the problem of quenching and should be protected from light during storage and operation.
2. The product is liquid at a concentration of 5 mg/mL.
3. Product expiration date: 6 months at 4°C.
4. Buffers can refer to PBS, HHBS, etc., but it is necessary to pay attention to the possible reactions of phosphate ions and calcium ions.
5. This product is for scientific research use only.



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