

## Image-iT™ LIVE Plasma Membrane and Nuclear Labeling Kit (I34406)

### Quick Facts

#### Storage upon receipt:

- ≤–20°C
- Protect from light
- Desiccate
- Avoid freeze-thaw cycles

#### Ex/Em:

- 591/618 nm (Alexa Fluor® 594 wheat germ agglutinin)
- 350/461 nm (Hoechst 33342 dye)

**Number of Assays:** 360

### Introduction

The Image-iT™ LIVE Plasma Membrane and Nuclear Labeling Kit provides two stains — red-fluorescent Alexa Fluor® 594 wheat germ agglutinin (WGA) and blue-fluorescent Hoechst 33342 dye — for highly selective staining of the plasma membrane and nucleus, respectively, of live green-fluorescent protein (GFP)–transfected cells. Using the protocol provided, the dyes can be combined into one staining solution to save labeling time and wash steps while still providing optimal staining.

Cell-impermeant Alexa Fluor 594 WGA binds selectively to *N*-acetylglucosamine and *N*-acetylneuraminic (sialic) acid residues.<sup>1</sup> When used according to the protocol, Alexa Fluor 594 WGA provides highly selective labeling of the plasma membrane with minimal background, although labeling may not be as distinct for flat cell types when viewed using standard epifluorescence microscopy and/or low magnification. Alexa Fluor 594 WGA is retained after formaldehyde fixation and permeabilization with Triton X-100. This fluorescent lectin conjugate can also be used to label fixed cells; however, to avoid labeling intracellular components, formaldehyde-fixed cells should not be permeabilized before labeling. It is important to note that Alexa Fluor 594 WGA can stimulate biological activity, including clustering of glycosylated cell-surface proteins.

The kit also includes Hoechst 33342 dye, a cell-permeant nucleic acid stain that is selective for DNA and is spectrally similar to DAPI. Hoechst 33342 dye is UV excitable and emits blue fluorescence when bound to DNA. This dye should not interfere with GFP fluorescence and is retained after fixation and permeabilization.

### Materials

#### Kit Contents

- **Wheat germ agglutinin, Alexa Fluor 594 conjugate** (Component A), 2.0 mg
- **Hoechst 33342 dye** (Component B), 3 vials, each containing 400 µL at 1.0 mM in water

#### Storage and Handling

Upon receipt, the kit should be stored upright, desiccated, and protected from light at ≤–20°C. Avoid freeze-thaw cycles. Vials should be allowed to warm to room temperature before opening. When stored properly, components should be stable for at least 6 months.

#### Spectral Characteristics

Alexa Fluor 594 WGA has excitation/emission maxima of approximately 591/618 nm, and Hoechst 33342 dye has excitation/emission maxima of approximately 350/461 nm. Cells labeled with Alexa Fluor 594 WGA and Hoechst 33342 dye can be imaged using standard filter sets.

#### Materials Recommended but Not Provided

Hank's balanced salt solution (HBSS, available from Gibco (14025-092)).

### Experimental Protocol

#### Reagent Preparation

##### Prepare 1.0 mg/mL Alexa Fluor 594 WGA stock solution.

Dissolve the 2.0 mg of lyophilized Alexa Fluor 594 wheat germ agglutinin (Component A) in 2.0 mL of phosphate-buffered saline (PBS) or water to make a 1.0 mg/ml stock solution.

#### Labeling Live Eukaryotic Cells

This is a general procedure for labeling live, cultured cells that are adhering to coverslips. The protocol was optimized using HBSS for HeLa cells transfected with GFP. Alexa Fluor 594 WGA and Hoechst 33342 dye are combined into one solution for single-step staining, but the two dyes can be used in separate labeling steps if desired. Recommended times and concentrations may vary in different model systems and may require optimization.

**1.1 Prepare labeling solution.** Dilute the 1.0 mg/mL Alexa Fluor 594 WGA stock solution (prepared above) and the 1.0 mM Hoechst 33342 dye (Component B) into HBSS. A recommended concentration for Alexa Fluor 594 WGA is

5.0 µg/mL; a recommended concentration for Hoechst 33342 stain is in the range of 1–2 µM. Using cell-culture medium to dilute WGA conjugates for labeling may cause increased off-cell background.

**1.2 Label cells.** Apply a sufficient amount of labeling solution to cover cells adhering to coverslip(s). Incubate for 10 minutes at 37°C.

**1.3 Wash cells.** When labeling is complete, remove the labeling solution and wash cells twice in suitable buffer. Unless the cells will be fixed, samples are ready to mount in warm HBSS or suitable buffer for imaging.

**1.4 (Optional) Fix cells.** Labeled cells can be fixed with 4% formaldehyde for 15 minutes at 37°C, followed by washes in buffer and any additional counterstains. Cells may also be permeabilized as necessary with 0.2% Triton X-100 (after labeling with Alexa Fluor 594 wheat germ agglutinin).

### **Labeling Fixed Eukaryotic Cells**

This protocol was optimized for formaldehyde-fixed cells. Alexa Fluor 594 WGA and Hoechst 33342 dye are combined into one solution for single-step staining, but the two dyes can be used in separate labeling steps if desired. Recommended times and concentrations may vary in different model systems and may require optimization.

**2.1 Fix cells.** Fix cells with 4% formaldehyde for 15 minutes at 37°C.

**2.2 Wash cells.** Wash cells three times in HBSS. Do not permeabilize the cells.

**2.3 Prepare labeling solution.** Dilute the 1.0 mg/mL Alexa Fluor 594 wheat germ agglutinin stock solution (prepared above) and the 1.0 mM Hoechst 33342 stain (Component B) into HBSS. A recommended concentration for Alexa Fluor 594 wheat germ agglutinin is 5.0 µg/mL; a recommended concentration for Hoechst 33342 stain is in the range of 1–2 µM. Both dyes may be combined in a single staining solution.

**2.4 Label cells.** Apply a sufficient amount of labeling solution to cover cells adhering to coverslip(s). Incubate for 10 minutes at room temperature.

**2.5 Wash cells.** When labeling is complete, remove the labeling solution and wash cells twice in HBSS or suitable buffer.

**2.6 Prepare cells for viewing.** Stain the cells with additional counterstains as desired and mount in buffer or an antifade mounting medium such as ProLong antifade reagent (available in the ProLong Antifade Kit, P7481) or ProLong Gold antifade reagent (P36930).

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## **Reference**

1. J Mol Biol 178, 91 (1984).

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<b>Cat #</b>	<b>Product Name</b>	<b>Unit Size</b>
I34406	Image-iT™ LIVE Plasma Membrane and Nuclear Labeling Kit *counterstains for GFP-expressing cells* .....	1 kit

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### **Invitrogen European Headquarters**

Invitrogen, Ltd.  
3 Fountain Drive  
Inchinnan Business Park  
Paisley PA4 9RF, UK  
Phone: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260  
Email: [euroinfo@invitrogen.com](mailto:euroinfo@invitrogen.com)  
Technical Services: [eurotech@invitrogen.com](mailto:eurotech@invitrogen.com)

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