

Wheat Germ Agglutinin Conjugates

Table 1. Contents and storage information.

Material	Amount	Storage*	Stability
Wheat germ agglutinin (WGA), fluorescein conjugate	5 mg, lyophilized	<ul style="list-style-type: none"> • ≤−20°C • Desiccate • Protect from light • Avoid repeated freeze/thaw cycles 	When stored as directed, this kit is stable for at least 1 year.
WGA, tetramethylrhodamine conjugate			
WGA, Oregon Green® 488 conjugate			
WGA, Alexa Fluor® 350 conjugate			
WGA, Alexa Fluor® 488 conjugate			
WGA, Alexa Fluor® 555 conjugate			
WGA, Alexa Fluor® 594 conjugate			
WGA, Alexa Fluor® 633 conjugate			
WGA, Alexa Fluor® 647 conjugate			
WGA, Alexa Fluor® 680 conjugate	1 mg, lyophilized		
WGA, Texas Red®-X conjugate	1 mg each, lyophilized		
Wheat Germ Agglutinin Sampler Kit (Alexa Fluor® 350, Oregon Green® 488, tetramethylrhodamine, and Texas Red®-X)			

* Short-term exposure of wheat germ agglutinin (WGA) conjugates to dim light (e.g., room lighting) will not cause damage.

Approximate fluorescence excitation/emission maxima: See Table 2.

Introduction

Fluorescent lectins are versatile probes for detecting glycoconjugates in histochemical and flow cytometric applications and for localizing glycoproteins in gels. Wheat germ agglutinin selectively binds to N-acetylglucosamine and N-acetylneuraminic acid (sialic acid) residues.¹ In solution, wheat germ agglutinin exists as a heterodimer with a molecular weight of approximately 38,000 daltons and is normally cationic under physiological conditions. Molecular Probes® offers a broad selection of fluorescent wheat germ agglutinin conjugates. Table 1 provides a summary of peak excitation and emission wavelengths. The Wheat Germ Agglutinin Sampler Kit includes introductory samples of four fluorescent wheat germ agglutinin conjugates (Alexa Fluor® 350, Oregon Green® 488, tetramethylrhodamine, and Texas Red®-X conjugates).

Table 2. Fluorescent wheat germ agglutinin conjugates and their spectral characteristics.

Conjugate	Ex*	Em*
Alexa Fluor® 350	346	442
Fluorescein	494	518
Alexa Fluor® 488	495	519
Oregon Green® 488	496	524
Tetramethylrhodamine	555	580
Alexa Fluor® 555	555	565
Alexa Fluor® 594	590	617
Texas Red®-X	595	615
Alexa Fluor® 633	632	647
Alexa Fluor® 647	650	665
Alexa Fluor® 680	679	702

* Fluorescence excitation (Ex) and emission (Em) maxima, in nm.

Before Starting

Materials Required but Not Provided

- Phosphate-buffered saline (PBS), pH 7.4
- Hank's balanced salt solution (HBSS) without phenol red
- 0.2% Triton® X-100 in PBS
- Deionized water
- ProLong® Gold antifade reagent, if desired

Preparing Stock Solutions

To prepare a 1.0 mg/mL wheat germ agglutinin (WGA) conjugate stock solution, dissolve 5.0 mg of lyophilized WGA conjugate in 5.0 mL of phosphate-buffered saline (PBS) or water. You may store the stock solution at -20°C for at least a month. For short term storage, add sodium azide to a final concentration of 2 mM, and store at $2-6^{\circ}\text{C}$. **Protect from light, and avoid repeated freezing and thawing.**

Note: We recommend briefly centrifuging the protein conjugate solution before use, and only adding the supernatant to the experiment. This step eliminates any protein aggregates that may have formed in the solution, and thereby reduces nonspecific background staining. Typical working concentrations of wheat germ agglutinin conjugate solutions are 1–10 $\mu\text{g}/\text{mL}$.

Experimental Protocols for Selective Plasma Membrane Labeling

Labeling Live Eukaryotic Cells

This is a general procedure for labeling live, cultured cells that are adhering to coverslips. The protocol was optimized using Hank's balanced salt solution (HBSS) for mammalian cells. A number of different WGA conjugates have been validated with this protocol. Recommended times and concentrations may vary in different model systems and require optimization.

- 1.1 Prepare labeling solution.** Dilute the 1.0 mg/mL WGA conjugate stock solution (prepared above) into HBSS. We recommend a starting concentration of 5.0 µg/mL for WGA conjugates. 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 a suitable buffer. Unless the cells will be fixed, samples are ready to mount in pre-warmed HBSS or suitable buffer for imaging.
- 1.4 Optional: Fix cells.** You may fix labeled cells can with 4% formaldehyde for 15 minutes at 37°C, followed by washes in buffer and any additional counterstains. You may also permeabilize cells as necessary with 0.2% Triton® X-100.

Labeling Pre-Fixed Eukaryotic

Cells

This protocol was optimized for adherent, formaldehyde-fixed mammalian cells which have not yet been permeabilized. A number of different WGA conjugates have been validated with this protocol. Recommended times and concentrations may vary in different model systems and 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 WGA conjugate stock solution (prepared above) into HBSS. We recommended a WGA conjugate concentration of 5.0 µg/mL.
- 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 the cells twice in HBSS or suitable buffer. You may now permeabilize the cells with 0.2% Triton® X-100 or other detergent for subsequent counterstaining or antibody labeling.
- 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® Gold antifade reagent.

Reference

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

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat No.	Product Name	Unit Size
W834	wheat germ agglutinin, fluorescein conjugate	5 mg
W849	wheat germ agglutinin, tetramethylrhodamine conjugate	5 mg
W6748	wheat germ agglutinin, Oregon Green® 488 conjugate	5 mg
W11261	wheat germ agglutinin, Alexa Fluor® 488 conjugate	5 mg
W11262	wheat germ agglutinin, Alexa Fluor® 594 conjugate	5 mg
W11263	wheat germ agglutinin, Alexa Fluor® 350 conjugate	5 mg
W21404	wheat germ agglutinin, Alexa Fluor® 633 conjugate	5 mg
W21405	wheat germ agglutinin, Texas Red®-X conjugate	1 mg
W32464	wheat germ agglutinin, Alexa Fluor® 555 conjugate	5 mg
W32465	wheat germ agglutinin, Alexa Fluor® 680 conjugate	5 mg
W32466	wheat germ agglutinin, Alexa Fluor® 647 conjugate	5 mg
W7024	Wheat Germ Agglutinin Sampler Kit *four fluorescent conjugates, 1 mg each*	1 kit
Related Products		
10010-031	Phosphate buffered saline (PBS), pH 7.4 (1X), liquid	1,000 mL
14175-079	Hank's balanced salt solution (HBSS) with no calcium chloride, magnesium chloride, magnesium sulfate, or phenol red	1,000 mL
P36930	ProLong® Gold antifade reagent	10 mL

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