invitrogen USER GUIDE

Molecular Probes[™] Wheat Germ Agglutinin Conjugates

Catalog Numbers W11263, W11261, W32464, W11262, W21404, W32466, W32465, W56134, W834, W6748, W849, W21405, W7024, W56133, W56132

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product Description

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.

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.

Invitrogen[™] offers a broad selection of fluorescent wheat germ agglutinin conjugates. The Wheat Germ Agglutinin Sampler kit includes introductory samples of four fluorescent WGAs: Alexa Fluor[™] 350, Oregon Green[™] 488, tetramethylrhodamine, and Texas Red[™]-X conjugates.

Contents and Storage

Table 1 Wheat germ agglutinin (WGA) conjugates

Material	Cat. No.	Amount ^[1]	Fluorescence excitation / emission	Storage ^[2]
WGA, Alexa Fluor™ 350 conjugate	W11263	5 mg	346 nm / 442 nm	
WGA, Alexa Fluor™ Plus 405 conjugate	W56132	1 mg	408 nm / 450 nm	
WGA, Alexa Fluor™ 488 conjugate	W11261	5 mg	495 nm / 519 nm	
WGA, Alexa Fluor™ 555 conjugate	W32464	5 mg	555 nm / 580 nm	Store at ≤ 20°C. Desiccate. Avoid repeated freeze/thaw cycles. Protect from bright light. [3]
WGA, Alexa Fluor™ Plus 568 conjugate	W56133	1 mg	562 nm/ 583 nm	
WGA, Alexa Fluor™ 594 conjugate	W11262	5 mg	590 nm / 617 nm	
WGA, Alexa Fluor™ 633 conjugate	W21404	5 mg	632 nm/ 647 nm	
WGA, Alexa Fluor™ 647 conjugate	W32466	5 mg	650 nm/ 665 nm	
WGA, Alexa Fluor™ 680 conjugate	W32465	5 mg	679 nm / 702 nm	
WGA, Alexa Fluor™ Plus 770 conjugate	W56134	1 mg	770 nm / 797 nm	
WGA, fluorescein congugate	W834	5 mg	494 nm / 518 nm	
WGA, Oregon Green™ 488 conjugate	W6748	5 mg	496 nm / 524 nm	
WGA, tetramethylrhodamine conjugate	W849	5 mg	555 nm / 580 nm	
WGA, Texas Red™-X conjugate	W21405	1 mg	595 nm / 615 nm	
WGA Sampler Kit (Alexa Fluor™ 350, Oregon Green™ 488, tetramethylrhodamine, and Texas Red™-X)	W7024	1 mg each		

^[1] Conjugates are provided in a lyophilized format.



^[2] When stored as directed, this kit is stable for at least 1 year.

^[3] Short-term exposure of WGA conjugates to dim light (e.g. room lighting) will not cause damage.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

ltem	Source
Phosphate-buffered saline, PBS, pH 7.4, 1 X 1000 mL	10010-031
Hank's balanced salt solution, HBSS, with no calcium chloride, magnesium chloride, magnesium sulfate, or phenol red	14175-079
Image-iT™ Fixative Solution in PBS (4% formaldehyde, methanol-free)	FB002
Image-iT™ Paraformaldehyde 4% in 0.1 M Phosphate Buffer, 0.1 M Sodium Cacodylate Buffer (Methanol free), 10 x 20 mL	128800
Image-iT™ Paraformaldehyde 3%, Glutaraldehyde 0.35%, 10 x 20 mL	128900
Image-iT™ Glyoxal 3% Fixative	128700
Triton-X™-100 in PBS 0.2%	MLS
Deionized water	MLS
ProLong™ Glass Antifade Mountant, 5 x 2 mL	P36980
EVOS™ M7000 Imaging System	AMF7000

Prepare stock solution

To prepare a 1.0 mg/mL WGA conjugate stock solution:

- 1. Dissolve 5.0 mg of lyophilized WGA conjugate in 5.0 mL of phosphate buffered saline or water.
- 2. The stock solution may be stored 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°C.

Note: It is recommended that the protein conjugate solution is briefly centrifuged before use and that only the supernatant is added to the experiment. This step eliminates any protein aggregates that may have formed in the solutions, and reduces nonspecific background staining. Typical working concentrations for WGA conjugate solutions are 1-10 µg/mL.

IMPORTANT! Protect from light and avoid repeated freezing and thawing.

Selective plasma membrane labeling

Label 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. Dilute the 1.0 mg/mL WGA conjugate stock solution (see step 1 in the "Prepare stock solutions" section) into HBSS. A starting concentration of 5.0 µg/mL for WGA conjugates is recommended. Using cell-culture medium to dilute WGA conjugates for labeling may cause increased off-cell background.
- 2. Apply a sufficient amount of labeling solution to cover cells adhering to coverslip(s). Incubate for 10 minutes at 37°C.
- 3. When labeling is complete, remove the labeling solution and wash cells twice in a suitable buffer. Unless the cells will be fixed, they are ready to mount in pre-warmed HBSS or suitable buffer for imaging.
- 4. *Optional:* Cells may be fix labeled with 4% formaldehyde for 15 minutes at 37°C, followed by washes in buffer and any additional counter-stains. Cells may be permeabilized as necessary with 0.2% Triton-X[™]-100.

Label pre-fixed eukaryotic cells

This protocol was optimized for adherent, formaldehyde-fixed mammalian cells which have not 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.

- 1. Fix cells with 45 formaldehyde for 15 minutes at 37°C.
- 2. Wash cells three times in HBSS. Do not permeabilize the cells.
- 3. Dilute the 1.0 mg/mL WGA conjugate tock solution (see step 1 in the "Prepare stock solutions" section) into HBSS. A WGA conjugate concentration of 5.0 µg/mL is recommended.
- 4. Apply a sufficient amount of labeling solution to cover cells adhering to coverslip(s). Incubate for 10 minutes at room temperature.
- 5. When labeling is complete, remove the labeling solution, and wash the cells twice in HBSS or suitable buffer. Cells may be permeabilized with 0.2% Triton-X[™]-100 or other determent for subsequent counterstaining or antibody labeling.
- 6. Stain cells with additional counterstains as desired and mount in buffer or an antifade mounting medium such as ProLong[™] Glass antifade reagent.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision	Date	Description
A.0	15 February 2023	New document for Molecular Probes [™] Wheat Germ Agglutinin Conjugates.

The information in this guide is subject to change without notice.

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