

Product Information

Wheat Germ Agglutinin (WGA) Conjugates

Product List

Cat. No.	Conjugate	Unit Size	Ex/Em* (nm)
29021-1	CF@350 WGA	1 mg	350/450
29021		5 x 1 mg	
29027-1	CF@405S WGA	1 mg	411/431
29027		5 x 1 mg	
29028-1	CF@405M WGA	1 mg	416/452
29028		5 x 1 mg	
29022-1	CF@488A WGA	1 mg	490/516
29022		5 x 1 mg	
29064-1	CF@532 WGA	1 mg	531/552
29064		5 x 1 mg	
29076-1	CF@555 WGA	1 mg	554/568
29076		5 x 1 mg	
29077-1	CF@568 WGA	1 mg	562/584
29077		5 x 1 mg	
29023-1	CF@594 WGA	1 mg	593/615
29023		5 x 1 mg	
29024-1	CF@633 WGA	1 mg	629/650
29024		5 x 1 mg	
29026-1	CF@640R WGA	1 mg	642/663
29026		5 x 1 mg	
29029-1	CF@680 WGA	1 mg	681/698
29029		5 x 1 mg	
29025-1	CF@680R WGA	1 mg	680/701
29025		5 x 1 mg	
29059-1	CF@770 WGA	1 mg	770/797
29059		5 x 1 mg	
29073	HRP WGA	1 mg	N/A

* In PBS, pH 7.4

Storage and Handling

Store at -20°C, protected from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

Product Description

Wheat germ agglutinin (WGA) binds selectively to N-acetylglucosamine and sialic acid residues. WGA is commonly used to label glycoproteins for imaging of the plasma membrane in live or fixed cells or for western blotting. WGA also is useful as a retrograde or anterograde neuronal tracer. Staining with WGA conjugates may be tissue-type and cell-type dependent. WGA also labels gram-positive bacteria and yeast bud scars.

Biotium's next-generation fluorescent CF@ Dyes offer advantages in brightness and photostability compared to other fluorescent dyes. We also offer HRP WGA, which can be used with colorimetric HRP substrates or fluorescent tyramide substrates.

Experimental Protocols

Conjugate reconstitution

To prepare a 1 mg/mL stock solution, dissolve 1 mg of lyophilized WGA conjugate in 1 mL water. The lyophilized product contains enough PBS to yield a concentration of 1X PBS after reconstitution. The stock solution can be stored at 2-8°C for up to a week or stored in aliquots at -20°C for 12 months or longer. Sodium azide may be added to protect from bacterial contamination during long-term storage, however, do not use azide with HRP conjugates. Protect fluorescent conjugates from light.

Recommended solutions for staining cells

Bacterial cells:

0.15 M NaCl or 3 M KCl.

Yeast cells:

Synthetic defined complete (SDC) media, or Hank's balanced salt solution (HBSS) without phenol red. YPD media is not recommended due to autofluorescence.

Mammalian cells:

For fixed cells, PBS, or Hank's balanced salt solution (HBSS) without phenol red can be used. For live adherent cells, HBSS with calcium and magnesium is recommended as it maintains cell morphology. We have also performed staining in complete culture medium with serum with good results.

Protocols for staining live cells and fixed cells

These are general protocols for staining cells with WGA conjugates. Optimization for your tissue or cells of interest may be required. While WGA is commonly used for tissue staining, its localization is highly tissue-dependent, so it may not stain cell boundaries in your tissue of interest.

Materials required but not provided

- Paraformaldehyde, 4% in PBS (Cat. No. 22023).
- Permeabilization Buffer (Cat. No. 22016).

1. Staining live cells

- 1.1 Wash cells 2X with a recommended buffer.
- 1.2 Prepare a staining solution of WGA in a recommended buffer. Staining concentration may require optimization. Recommended starting concentration is 50-100 ug/mL for bacterial and yeast cells, and 1-5 ug/mL for mammalian cells.
- 1.3 Incubate cells with WGA staining solution, protected from light, for 10-30 minutes at room temperature for bacterial cells or 10 minutes at room temperature for mammalian cells.

Note: With longer incubation times, increased intracellular staining may be observed in mammalian cells.

- 1.4 Wash cells 2X with the same buffer or medium used for staining.

Notes:

- a. Washing with buffer such as HBSS or PBS is recommended before fixation; if free WGA is present during fixation and permeabilization, WGA will stain both cell surface and organelles in the secretory pathway. WGA staining is compatible with paraformaldehyde (PFA) and solvent fixation.
 - b. For live cell imaging, washing may be optional if live cells are being imaged by confocal, as the free WGA in solution may not interfere with imaging if you are focused on the cells. For epifluorescence, washing is highly recommended as free WGA can result in high background.
- 1.5 Image cells on a microscope using the appropriate filter set (see product table for peak excitation and emission). Staining also can be detected by flow cytometry in the appropriate detection channel.

2. Staining fixed cells

WGA staining is compatible with paraformaldehyde (PFA) fixation, solvent fixation, or FFPE tissue staining. However, using alcohol or solvent fixatives (like methanol or acetone) will cause more intracellular staining compared to PFA. Permeabilization of cells before staining will also cause WGA to label glycoproteins in the plasma membrane and intracellular compartments such as Golgi structures. Therefore, for cell surface labeling, we recommend using PFA fixation and staining with WGA prior to permeabilization.

- 2.1 Remove cell culture medium from live cells and rinse 3X with HBSS, PBS, or similar buffer.
- 2.2 Fix cells with 4% PFA in PBS for 20 minutes at room temperature.
Note: Avoid fixing the cells for longer than 20 minutes, as this can result in increased intracellular staining. Other fixation conditions (such as fixation with 2% PFA, or fixation at 4°C) may be optimal for preserving membrane integrity in your cell type.
- 2.3 Wash cells 3X with PBS.
- 2.4 Stain cells with CF® Dye WGA for 10-30 minutes at room temperature and protected from light. Recommended starting concentration is 1-5 ug/mL.
- 2.5 Wash cells 3X with PBS.
- 2.6 Permeabilize cells with 0.1% Triton® X-100 in PBS (or use Biotium's Permeabilization Buffer, Cat. No. 22016) for 30 minutes at room temperature and protected from light.
- 2.7 Wash cells 3X with PBS.
- 2.8 Proceed with immunofluorescence staining.
Note: We recommend omitting detergent from subsequent blocking and washing steps, if possible, to minimize potential redistribution or loss of WGA conjugate signal.
- 2.9 Image cells on a microscope using the appropriate filter set (see product table for peak excitation and emission).

Related Products

Cat. No.	Product
00070-00079	Cholera Toxin Subunit B CF® Dye Conjugates
29015-29080	CF® Dye Concanavalin A (Con A)
29060-29063	CF® Dye PNA Lectin (Arachis Hypogaea)
30021-30024	CelBrite® Cytoplasmic Membrane Dyes
30088-30090	CellBrite® Fix Membrane Stains
30105-30109	CellBrite® Steady Membrane Staining Kits
30092-30104	MemBrite® Fix Cell Surface Staining Kits
29067	Calcofluor White
40043	DAPI, 10 mg/mL in Water
40046	Hoechst 33342, 10 mg/mL in Water
32000-1	Live Bacterial Gram Stain Kit
32001	Bacterial Viability and Gram Stain Kit
30027	Viability/Cytotoxicity Assay Kit for Bacterial Live and Dead Cells
30015	DAB Substrate Kit
92170-96066	CF® Dye Tyramides
22027	Ready-to-Use Tyramide Amplification Buffer
22016	Permeabilization Buffer
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF® Dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology research.

CF® Dyes are covered by pending US and international patents. Triton® is a registered trademark of The Dow Chemical Company.

Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.