

Wheat Germ Agglutinin (WGA) Conjugates

AF660 Wheat Germ Agglutinin (WGA) Conjugates

Cat #: BGT-CHM-710

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 of 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, azide should not be used with HRP conjugates. Protect 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
- Permeabilization Buffer

Staining live cells

1. Wash cells 2X with a recommended buffer.
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.
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.
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.
5. Image cells on a microscope using the appropriate filter set (Ex/Em: 663/690nm). Staining also can be detected by flow cytometry in the appropriate detection channel.

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 intracellular compartments such as Golgi structures. Therefore, for cell surface labeling, we recommend using PFA fixation and staining with WGA prior to permeabilization.

1. Remove cell culture medium from live cells and rinse 3X with HBSS, PBS, or similar buffer.
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.

3. Wash cells 3X with PBS.
4. Stain cells with WGA for 10-30 minutes at room temperature and protected from light. Recommended starting concentration is 1-5 ug/mL.
5. Wash cells 3X with PBS.
6. Permeabilize cells with 0.1% Triton® X-100 in PBS for 30 minutes at room temperature and protected from light.
7. Wash cells 3X with PBS.
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.
9. Image cells on a microscope using the appropriate filter set (Ex/Em: 663/690nm).