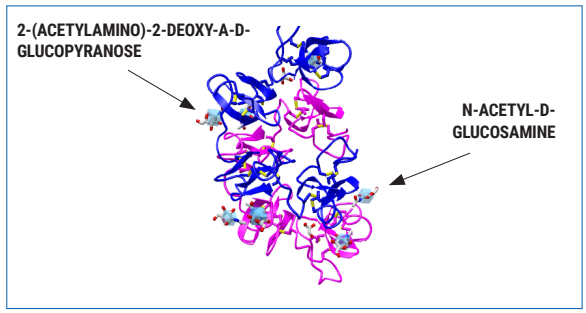
PRODUCT INFORMATION

PhenoVue Fluor - Wheat Germ Agglutinin Conjugates

Overview

Wheat germ agglutinin (WGA) is a lectin also known as carbohydrate binding protein. WGA displays high affinity for sialic acid and N-acetylglucosamine residues of glycoproteins and glycolipids present at the cellular plasma membranes. Therefore, fluorescent WGA conjugates represent a method of choice for labelling the cellular membranes of mammalian cells, particularly Golgi apparatus which is glycoprotein-enriched.



Structure of the Dimer Wheat Germ Agglutinin in Complex with N-Acetyl-D-Glucosamine. Source: PBD ID 2UVO

Product Information

Product Name	Part Number	Number of Vials per Unit	Quantity per Vial	Format	Shipping Conditions
PhenoVue Fluor 488 - WGA	CP14881	5	1 mg (29.2 nmoles)	Lyophilized	RT
PhenoVue Fluor 555 - WGA	CP15551	5	1 mg (29.2 nmoles)	Lyophilized	RT
PhenoVue Fluor 568 - WGA	CP15681	5	1 mg (29.2 nmoles)	Lyophilized	RT
PhenoVue Fluor 594 - WGA	CP15941	5	1 mg (29.2 nmoles)	Lyophilized	RT
PhenoVue Fluor 647 - WGA	CP16471	5	1 mg (29.2 nmoles)	Lyophilized	RT

Storage and Stability

- Store lyophilized reagents at 2-8 °C, protected from light.
- The stability of these products is guaranteed until the expiration date provided in the Certificate of Analysis, when stored as recommended and protected from light.
- Allow the powder to warm up to room temperature for 15 min before opening the vials and reconstitution.
- After reconstitution, aliquoted reagents must be stored at -16 °C or below and are stable for 6 months. Avoid repeated freeze / thaw cycles.

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Recommended Reconstitution

Product Name	Molecular Weight	Recommended Stock Concentration	Working Concentration Range*
PhenoVue Fluor 488 - WGA	34300 g/mol	Reconstitution using 1 mL ddH_2O gives a stock concentration of 1 mg/mL (29.2 µM)	1 µg/mL - 10 µg/mL (29.2 nM - 292 nM)
PhenoVue Fluor 555 - WGA	34300 g/mol	Reconstitution using 1 mL ddH_2O gives a stock concentration of 1 mg/mL (29.2 µM)	1 µg/mL - 10 µg/mL (29.2 nM - 292 nM)
PhenoVue Fluor 568 - WGA	34300 g/mol	Reconstitution using 1 mL ddH_2O gives a stock concentration of 1 mg/mL (29.2 µM)	1 µg/mL - 10 µg/mL (29.2 nM - 292 nM)
PhenoVue Fluor 594 - WGA	34300 g/mol	Reconstitution using 1 mL ddH_2O gives a stock concentration of 1 mg/mL (29.2 µM)	1 µg/mL - 10 µg/mL (29.2 nM - 292 nM)
PhenoVue Fluor 647 - WGA	34300 g/mol	Reconstitution using 1 mL ddH_2O gives a stock concentration of 1 mg/mL (29.2 µM)	1 µg/mL - 10 µg/mL (29.2 nM - 292 nM)

* Dilutions can be done in HBSS, PhenoVue dye diluent A or PBS.

Equivalent Number of Microplates

Product Name	When Used at Recommended Concentration	96-well Microplate (100 µL - 300 µL per Well)	384-well Microplate (25 µL - 90 µL per Well)	1536-well Microplate (4 µL - 12 µL per Well)
PhenoVue Fluor 488 - WGA	5 µg/mL (146 nM)	Approx. 35 to 100	Approx. 30 to 100	Approx. 55 to 160
PhenoVue Fluor 555 - WGA	5 µg/mL (146 nM)	Approx. 35 to 100	Approx. 30 to 100	Approx. 55 to 160
PhenoVue Fluor 568 - WGA	5 µg/mL (146 nM)	Approx. 35 to 100	Approx. 30 to 100	Approx. 55 to 160
PhenoVue Fluor 594 - WGA	5 µg/mL (146 nM)	Approx. 35 to 100	Approx. 30 to 100	Approx. 55 to 160
PhenoVue Fluor 647 - WGA	5 µg/mL (146 nM)	Approx. 35 to 100	Approx. 30 to 100	Approx. 55 to 160

See PerkinElmer's range of high-quality imaging microplates here: www.perkinelmer.com/category/microplates-imaging

Spectral and Photophysical Properties

Product Name	Maximum Excitation Wavelength (nm)	Maximum Emission Wavelength (nm)	Common Filters Set	Quantum Yield (Φ)	Epsilon* (ε in $M^{-1}.cm^{-1}$ at λ max)	Brightness (Φ x ε)
PhenoVue Fluor 488 - WGA	495	520	FITC	92%	73000	65320
PhenoVue Fluor 555 - WGA	555	570	Cy3	10%	155000	15500
PhenoVue Fluor 568 - WGA	578	603	Texas-Red	69%	88000	60720
PhenoVue Fluor 594 - WGA	590	617	Texas-Red	66%	92000	60720
PhenoVue Fluor 647 - WGA	650	670	Cy5	30%	240000	72000

* In PBS or HBSS pH 7.4

Live and Fixed-Cell Compatibility

Product Name	Live-Cell Staining	Fixation/Permeabilization Steps Post Live-Cell Staining	Fixed-Cell Staining
PhenoVue Fluor 488 - WGA	Yes	Yes	Yes
PhenoVue Fluor 555 - WGA	Yes	Yes	Yes
PhenoVue Fluor 568 - WGA	Yes	Yes	Yes
PhenoVue Fluor 594 - WGA	Yes	Yes	Yes
PhenoVue Fluor 647 - WGA	Yes	Yes	Yes

Protocols

Cell Culture

Seed cells in imaging microplates (or any other convenient cell culture vessels). Incubate in the appropriate cell culture conditions, usually 37 °C, 5% CO_2 until 50-70% confluency.

PhenoVue Fluor – WGA conjugates are not cell-permeable, therefore fixed but non-permeabilized cells exhibit plasma membrane staining. An additional permeabilization step enables staining of cytoplasmic membranes such as Golgi apparatus.

Fixed-Cell Imaging

1. **Rinse** briefly in phosphate-buffered saline (PBS) then proceed with cell fixation.
2. **Fixation:** 2 options:
    1. Add ready to use PhenoVue Paraformaldehyde 4% Methanol-Free Solution (PVPFA41) for 10 min at room temperature. Note that paraformaldehyde (PFA) is the most popular fixative reagent.

       or

    2. Add 100% methanol (chilled to - 20 °C) at room temperature for 5 min.
3. **Washing:** Wash three times with PBS.
4. **Permeabilization:**
    1. For PFA fixed cells, add ready to use PhenoVue Permeabilization 0.5% Triton X-100 Solution (PVPERM051) for 10 min (for membrane-associated antigens, 100 µM digitonin or 0.5% saponin are preferred). Triton X-100 is the most popular detergent for improving the penetration of antibodies. However, it may not be appropriate for some imaging applications since it can destroy membranes.
    2. Methanol fixed cells do not require permeabilization.
5. **Washing:** Wash three times with PBS for 5 min.
6. **Incubate:** Incubate with 1-10 µg/mL PhenoVue Fluor – WGA conjugates diluted in HBSS for 10-60 min at RT.
7. **Washing:** Wash three times with PBS for 5 min.
8. **Optional:** Incubate with 1-5 µg/mL PhenoVue Hoechst 33342 nuclear stain for 10 min.
9. **Washing:** Wash once with PBS for 5 min.
10. Acquire images on an imaging device.

Live-Cell Imaging

PhenoVue Fluor - WGAs stain plasma membrane and eventually intracellular vesicles after invagination of the plasma membrane.

1. Rinse briefly in HBSS.
2. Incubate with 1-10 µg/mL PhenoVue Fluor – WGA conjugates diluted in HBSS for 10-60 min at RT.
3. Rinse in HBSS.
4. Acquire images on a live-cell imaging device.

Note that cytotoxicity of staining reagents such as Hoechst 33342 is usually observed in long term imaging.

Tips

- To remove protein aggregates that can form during storage, spin down PhenoVue Fluor – WGA conjugates to prepare working solution. It may help to reduce nonspecific background.
- The homodimer WGA structure binds 4 to 8 carbohydrate moieties (Portillo-Tellez Biophysicol J 2011; Schwefel D et al., J Am Chem Soc 2010).
- At neutral pH, WGA forms dimers which dissociate into monomers at lower pH. Moreover, WGA tends to aggregate at higher pH (> 8). For reproducible and accurate results, pH of staining buffers should be controlled and ideally kept in neutral range (7-7.4).
- The composition of PhenoVue dye diluent A (part number PVDDA1) has been optimized to maximize staining efficacy.
- PhenoVue Fluor – WGA conjugates are not cell-permeable, therefore fixed but non-permeabilized cells exhibit plasma membrane staining, whereas additional permeabilization step enables staining of cytoplasmic membranes such as Golgi apparatus.
- In live-cell imaging experiments, PhenoVue Fluor – WGA conjugates stain plasma membrane and eventually intracellular vesicles after invagination of the plasma membrane.

Safety Information

Chemical reagents are potentially harmful, please refer to the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Applications

- High-Content Analysis / High-Content Screening
- Microscopy
- Cytometry

Validation Data

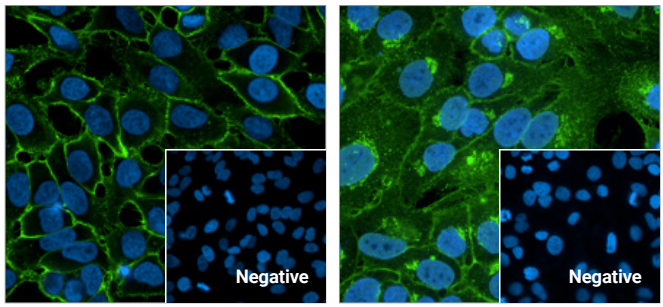

*Figure 1: HeLa cells were seeded in PhenoPlate™ 96-well microplates (50,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were either fixed (A) or fixed and permeabilized (B), then stained with 5 µg/mL of **PhenoVue Fluor 488 - WGA** for 10 minutes at RT. Unlike plasma membranes which are specifically stained after fixation, intracellular membranes like Golgi apparatus are detected after permeabilization. Background staining (inset negative condition) is obtained by pre-incubating non-fluorescent WGA (100X, 30min) before the addition of the PhenoVue Fluor 488 - Concanavalin A. Images were acquired on the Operetta™ CLS high-content analysis system.*

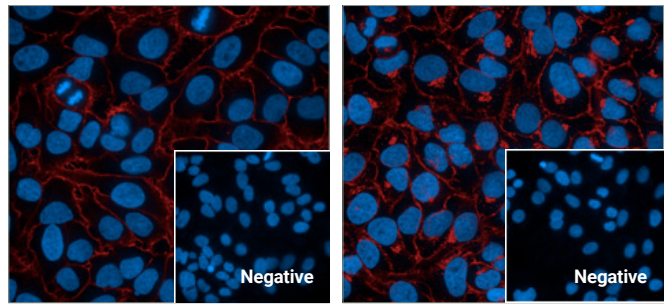

*Figure 2: HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were either fixed (A) or fixed and permeabilized (B), then stained with 5 µg/mL of **PhenoVue Fluor 594 - WGA** for 10 minutes at RT. Background staining (inset negative images) is obtained by pre-incubating non fluorescent WGA (100X) before the PhenoVue Fluor 488 - WGA. Unlike plasma membranes which are specifically stained after fixation (A), intracellular membranes like Golgi apparatus are detected after permeabilization (B). Images were acquired on the Operetta CLS high-content analysis system.*

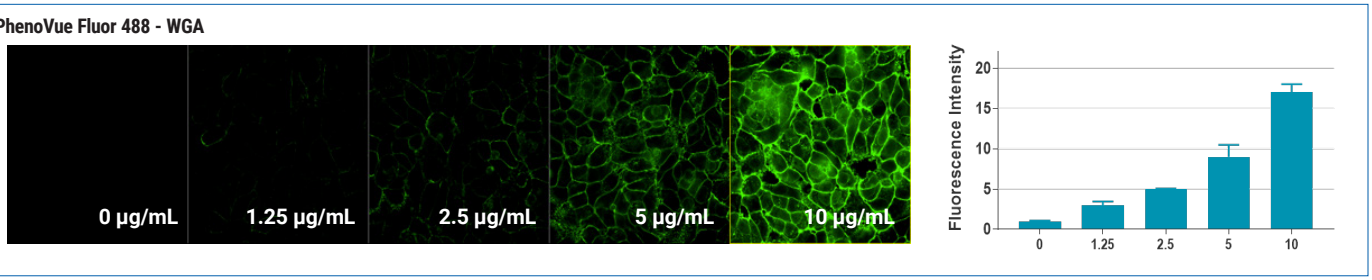

*Figure 3: HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed but not permeabilized and stained with increasing concentrations of **PhenoVue Fluor 488 - WGA** for 10 minutes at RT. Images were acquired on the Operetta CLS high-content analysis system.*

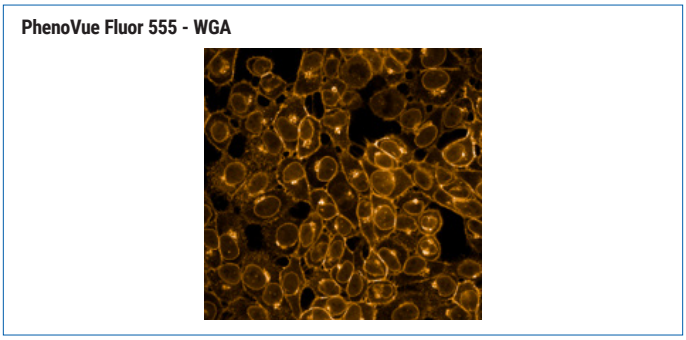

*Figure 4: HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and stained with 5 µg/mL of **PhenoVue Fluor 555 - WGA** for 10 min at RT. Images were acquired on the Operetta CLS high-content analysis system.*

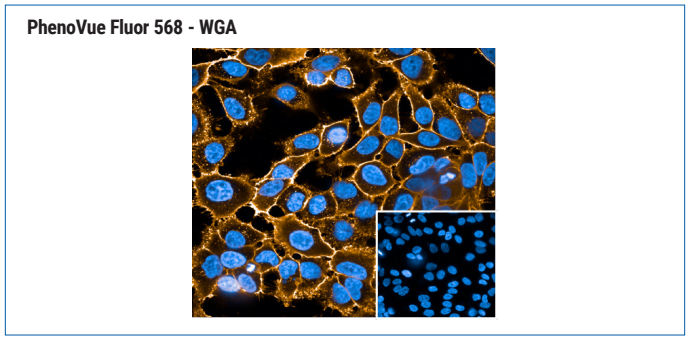

*Figure 5 HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed and stained with 5 µg/mL of **PhenoVue Fluor 568 - WGA** for 10 min at RT. Background staining (inset negative condition) is obtained by pre-incubating non-fluorescent WGA (100X, 30min) before the PhenoVue Fluor 568 - Concanavalin A. Images were acquired on the Operetta CLS high-content analysis system.*

Validation Data

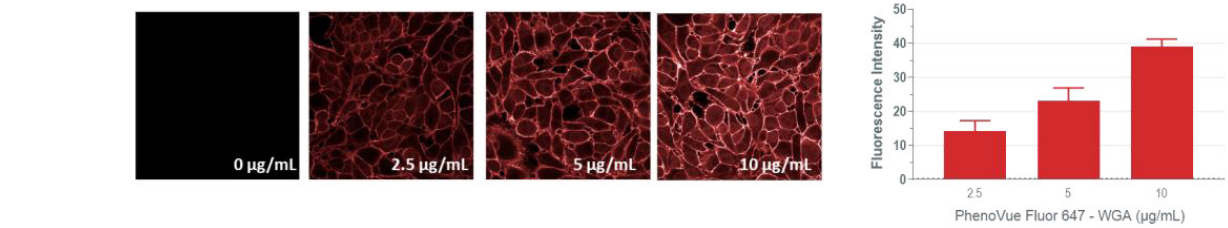

*Figure 6: HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed (but not permeabilized) and stained with increasing concentrations of **PhenoVue Fluor 647 - WGA** for 10 min at RT. Images were acquired on the Operetta CLS high-content analysis system.*

Related Products

Opera Phenix® Plus High-Content Screening System
www.perkinelmer.com/operaphenixplus

Operetta® CLS™ High-Content Analysis System
www.perkinelmer.com/operettaCLS

Harmony® Imaging and Analysis Software
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PhenoPlate high-quality microplates for imaging
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PhenoVue Cell Painting Kits
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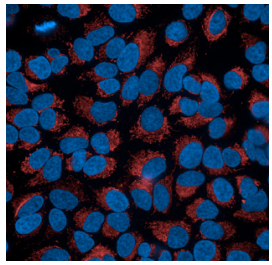

*Figure 7: HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24 h. Live cells were stained with 150 nM of **PhenoVue Fluor 641 Mitochondrial stain** for 30 min at 37 °C prior to fixation and permeabilization. Images were acquired on the Operetta CLS high-content analysis system.*

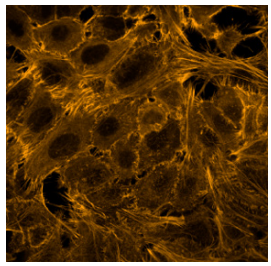

*Figure 8: HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24 h. Cells were fixed then permeabilized and stained with 165 nM of **PhenoVue Fluor 568 - Phalloidin** for 45 min at RT. Images were acquired on the Operetta CLS high-content analysis system.*

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