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.

### Product information

<table>
<thead>
<tr>
<th>Product name</th>
<th>Part no.</th>
<th>Number of vials per unit</th>
<th>Quantity per vial</th>
<th>Format</th>
<th>Shipping conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhenoVue Fluor 488 - WGA</td>
<td>CP14881</td>
<td>5</td>
<td>1 mg (29.2 nmoles)</td>
<td>Lyophilized</td>
<td>RT</td>
</tr>
<tr>
<td>PhenoVue Fluor 555 - WGA</td>
<td>CP15551</td>
<td>5</td>
<td>1 mg (29.2 nmoles)</td>
<td>Lyophilized</td>
<td>RT</td>
</tr>
<tr>
<td>PhenoVue Fluor 568 - WGA</td>
<td>CP15681</td>
<td>5</td>
<td>1 mg (29.2 nmoles)</td>
<td>Lyophilized</td>
<td>RT</td>
</tr>
<tr>
<td>PhenoVue Fluor 594 - WGA</td>
<td>CP15941</td>
<td>5</td>
<td>1 mg (29.2 nmoles)</td>
<td>Lyophilized</td>
<td>RT</td>
</tr>
<tr>
<td>PhenoVue Fluor 647 - WGA</td>
<td>CP16471</td>
<td>5</td>
<td>1 mg (29.2 nmoles)</td>
<td>Lyophilized</td>
<td>RT</td>
</tr>
</tbody>
</table>

### 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.
Recommended reconstitution

<table>
<thead>
<tr>
<th>Product name</th>
<th>Molecular weight</th>
<th>Recommended stock concentration</th>
<th>Working concentration range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhenoVue Fluor 488 - WGA</td>
<td>34300 g/mol</td>
<td>Reconstitution using 1 mL ddH$_2$O gives a stock concentration of 1 mg/mL (29.2 µM)</td>
<td>1 µg/mL - 10 µg/mL (29.2 nM - 292 nM)</td>
</tr>
<tr>
<td>PhenoVue Fluor 555 - WGA</td>
<td>34300 g/mol</td>
<td>Reconstitution using 1 mL ddH$_2$O gives a stock concentration of 1 mg/mL (29.2 µM)</td>
<td>1 µg/mL - 10 µg/mL (29.2 nM - 292 nM)</td>
</tr>
<tr>
<td>PhenoVue Fluor 568 - WGA</td>
<td>34300 g/mol</td>
<td>Reconstitution using 1 mL ddH$_2$O gives a stock concentration of 1 mg/mL (29.2 µM)</td>
<td>1 µg/mL - 10 µg/mL (29.2 nM - 292 nM)</td>
</tr>
<tr>
<td>PhenoVue Fluor 594 - WGA</td>
<td>34300 g/mol</td>
<td>Reconstitution using 1 mL ddH$_2$O gives a stock concentration of 1 mg/mL (29.2 µM)</td>
<td>1 µg/mL - 10 µg/mL (29.2 nM - 292 nM)</td>
</tr>
<tr>
<td>PhenoVue Fluor 647 - WGA</td>
<td>34300 g/mol</td>
<td>Reconstitution using 1 mL ddH$_2$O gives a stock concentration of 1 mg/mL (29.2 µM)</td>
<td>1 µg/mL - 10 µg/mL (29.2 nM - 292 nM)</td>
</tr>
</tbody>
</table>

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

Equivalent number of microplates

<table>
<thead>
<tr>
<th>Product name</th>
<th>When used at recommended concentration</th>
<th>96-well microplate (100 µL - 300 µL per well)</th>
<th>384-well microplate (25 µL - 90 µL per well)</th>
<th>1536-well microplate (4 µL - 12 µL per well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhenoVue Fluor 488 - WGA</td>
<td>5 µg/mL (146 nM)</td>
<td>Approx. 35 to 100</td>
<td>Approx. 30 to 100</td>
<td>Approx. 55 to 160</td>
</tr>
<tr>
<td>PhenoVue Fluor 555 - WGA</td>
<td>5 µg/mL (146 nM)</td>
<td>Approx. 35 to 100</td>
<td>Approx. 30 to 100</td>
<td>Approx. 55 to 160</td>
</tr>
<tr>
<td>PhenoVue Fluor 568 - WGA</td>
<td>5 µg/mL (146 nM)</td>
<td>Approx. 35 to 100</td>
<td>Approx. 30 to 100</td>
<td>Approx. 55 to 160</td>
</tr>
<tr>
<td>PhenoVue Fluor 594 - WGA</td>
<td>5 µg/mL (146 nM)</td>
<td>Approx. 35 to 100</td>
<td>Approx. 30 to 100</td>
<td>Approx. 55 to 160</td>
</tr>
<tr>
<td>PhenoVue Fluor 647 - WGA</td>
<td>5 µg/mL (146 nM)</td>
<td>Approx. 35 to 100</td>
<td>Approx. 30 to 100</td>
<td>Approx. 55 to 160</td>
</tr>
</tbody>
</table>

View our full range of high-quality imaging microplates at Revvity.com

Spectral and photophysical properties

<table>
<thead>
<tr>
<th>Product name</th>
<th>Maximum excitation wavelength (nm)</th>
<th>Maximum emission wavelength (nm)</th>
<th>Common filter set</th>
<th>Quantum yield (Φ)</th>
<th>Epsilon* (ε in M$^{-1}$.cm$^{-1}$ at λ max)</th>
<th>Brightness (Φ x ε)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhenoVue Fluor 488 - WGA</td>
<td>495</td>
<td>520</td>
<td>FITC</td>
<td>92%</td>
<td>73000</td>
<td>65320</td>
</tr>
<tr>
<td>PhenoVue Fluor 555 - WGA</td>
<td>555</td>
<td>570</td>
<td>Cy3</td>
<td>10%</td>
<td>155000</td>
<td>15500</td>
</tr>
<tr>
<td>PhenoVue Fluor 568 - WGA</td>
<td>578</td>
<td>603</td>
<td>Texas-Red</td>
<td>69%</td>
<td>88000</td>
<td>60720</td>
</tr>
<tr>
<td>PhenoVue Fluor 594 - WGA</td>
<td>590</td>
<td>617</td>
<td>Texas-Red</td>
<td>66%</td>
<td>92000</td>
<td>60720</td>
</tr>
<tr>
<td>PhenoVue Fluor 647 - WGA</td>
<td>650</td>
<td>670</td>
<td>Cy5</td>
<td>30%</td>
<td>240000</td>
<td>72000</td>
</tr>
</tbody>
</table>

* In PBS or HBSS pH 7.4

Live and fixed-cell compatibility

<table>
<thead>
<tr>
<th>Product name</th>
<th>Live-cell staining</th>
<th>Fixation/permeabilization steps post live-cell staining</th>
<th>Fixed-cell staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhenoVue Fluor 488 - WGA</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>PhenoVue Fluor 555 - WGA</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>PhenoVue Fluor 568 - WGA</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>PhenoVue Fluor 594 - WGA</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>PhenoVue Fluor 647 - WGA</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
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% CO₂ 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.

**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.
- 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% CO₂ 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, 30 min) 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% CO₂ 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% CO₂ 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% CO2 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% CO2 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.

Figure 6: HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO2 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.