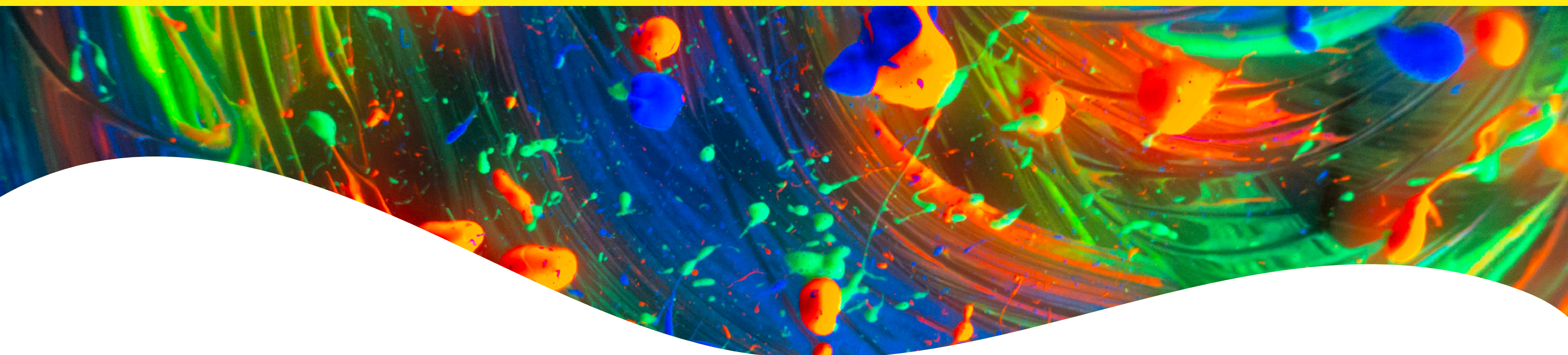
revvity

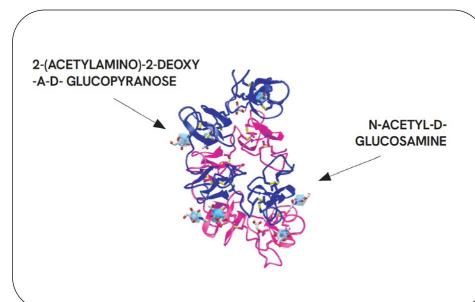
PhenoVue Fluor – WGA Conjugates



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 resent 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 no.	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 400LS - WGA	CP14001	2	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.

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 400LS - WGA	34300 g/mol	Reconstitution using 1 mL ddH_2O gives a stock concentration of 1 mg/mL (29.2 µM)	5 µg/mL – 50 µg/mL (146 nM – 1460 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)	384-well microplate (25 µL - 90 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 400LS - WGA	25 µg/mL (730 nM)	Approx. 2.6 to 8	Approx. 2 to 8	Approx. 4 to 13
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

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

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 400LS - WGA	395	585	Ex: 375-440 Em: 550-650	nd	26000	nd
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 pH 7.4
nd: not determined

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 400LS – 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.

Note: 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.

2. Washing: Wash three times with PBS.

3. **Permeabilization (for Golgi Apparatus staining):**

    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

4. **Washing:** Wash three times with PBS for 5 min.

5. **Staining:** Incubate with 1-50 µg/mL PhenoVue Fluor - WGA conjugates diluted in HBSS or PhenoVue Dye Diluent A for 10-60 min at RT*.

6. **Washing:** Wash three times with PBS for 5 min.

7. **Optional:** Incubate with 0.02-5 µg/mL PhenoVue Hoechst 33342 nuclear stain for 10 min or more. Alternatively, PhenoVue Hoechst 33342 nuclear stain can be mixed with the PhenoVue Fluor-WGA conjugates staining solutions.

8. **Washing:** Wash once with PBS for 5 min.

9. **Acquire** images on an imaging device

*See recommended concentrations in the table "recommended reconstitution and Equivalent number of microplates"

Live-cell imaging

Note: PhenoVue Fluor - WGA conjugates stain the plasma membrane and eventually intracellular vesicles after invagination of the plasma membrane.

1. Rinse briefly in HBSS.

2. Incubate with 1-50 µg/mL PhenoVue Fluor WGA in HBSS or PhenoVue Dye Diluent A for 10-60 min at RT*. PhenoVue Hoechst 33342 nuclear stain (0.02-5 µg/mL) can be mixed for nucleus staining if necessary.

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.

*See recommended concentrations in the table "recommended reconstitution and Equivalent number of microplates"

Tips

- To remove protein aggregates that can form during storage, spin down PhenoVue Fluor - WGA conjugates to prepare a working solution. It may help to reduce non-specific background.

- The homodimeric WGA structure can bind 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 an additional permeabilization step is required for 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 membranes.

Special recommendations for PhenoVue Fluor 400LS – WGA in a 5-plex experiment

PhenoVue Fluor 400LS - WGA is a long Stokes shift dye which allows multiplexing of up to 5 colors.

To obtain a high fluorescent signal, please note the following acquisition settings:

- **Excitation of PhenoVue Fluor 400LS between 360 and 415 nm (e.g. Opera Phenix™/Plus with 405 nm or Operetta CLS™ with 405 or 365 nm excitation):**
  - Reduce the concentration of Hoechst 33342 (or DAPI) to limit its crosstalk to the 570-630 nm detection band. A Hoechst (or DAPI) concentration of 20 - 80 ng/mL (incubated for 30-60 min) typically gives good nuclear staining while significantly reducing the crosstalk.
- **Excitation of PhenoVue Fluor 400LS with greater than 415 nm (e.g. Operetta CLS with 440 nm excitation):**
  - When used together with PhenoVue Fluor 488 conjugates, use a 600-640 nm emission band for PhenoVue Fluor 400LS to limit the crosstalk of PhenoVue Fluor 488.
- **For simultaneous acquisition (e.g. Opera Phenix/Plus):**
  - Separate Hoechst 33342 (Ex: 405/425 nm, Em: 435-480 nm) and PhenoVue Fluor 555/568 (Ex: 561 nm; Em: 570-630 nm) channels. 405 or 425 nm excitation of PhenoVue Fluor 400LS – WGA may result in an emission in the 570-630 nm detection band.

HCS Instruments		PhenoVue Hoechst 33342	PhenoVue Fluor 400LS	PhenoVue Fluor 488	PhenoVue Fluor 555 or Fluor 568	PhenoVue Fluor 647
Opera Phenix Plus 5 lasers	Excitation laser	375	425	488	561	640
	Emission filter	435-480	570-630	500-550	570-630	650-760
Opera Phenix Plus 4 lasers	Excitation laser	405	405	488	561	640
	Emission filter	435-480	570-630	500-550	570-630	650-760
Operetta CLS 8 LED - 1600	Excitation LED (filter)	370 (355-385)	405 (390-420)	475 (460-490)	550 (530-560)	630 (615-645)
	Emission filter	430-500	570-650	500-550	570-650	655-760
Operetta CLS 8 LED - 1601	Excitation LED (filter)	370 (355-385)	440 (435-460)	475 (460-490)	550 (530-560)	630 (615-645)
	Emission filter	430-500	600- 640 or 570-650	500-550	570-650	655-760
Operetta CLS 4 LED	Excitation LED (filter)	370 (355-385)	370 (355-385)	475 (460-490)	550 (530-560)	630 (615-645)
	Emission filter	430-500	570-650	500-550	570-650	655-760

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
- Imaging microscopy
- Cytometry

Validation data

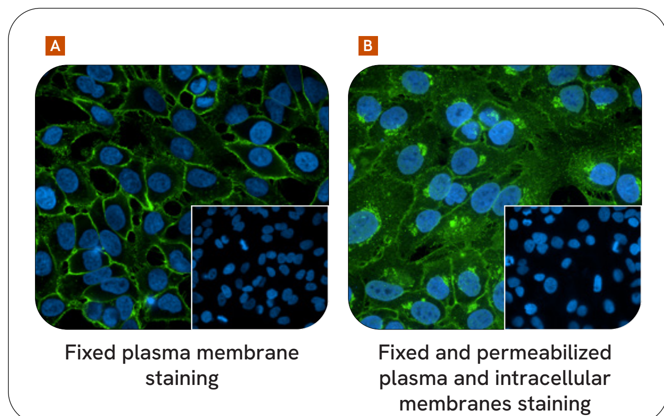


Figure 1: HeLa cells were seeded in PhenoPlate™ 96-well microplates (50,000 cells/ well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were either fixed **(A)** or fixed and permeabilized **(B),** then stained with 5 µg/mL of **PhenoVue Fluor 488 - WGA** for 10 minutes at RT. Unlike plasma membranes 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, 30 min) before the addition of the PhenoVue Fluor 488 - Concanavalin A. Images were acquired on the Operetta CLS high-content analysis system.

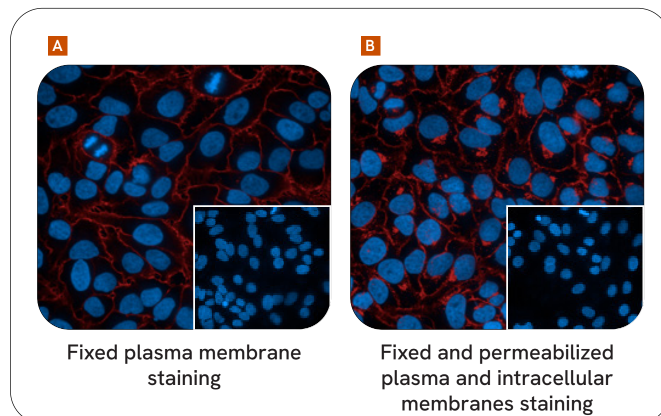


Figure 2: HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/ well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were either fixed (A) or fixed and permeabilized (B), then stained with 5 µg/mL of **PhenoVue Fluor 594 - WGA** for 10 minutes at RT. Background staining (inset negative 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.

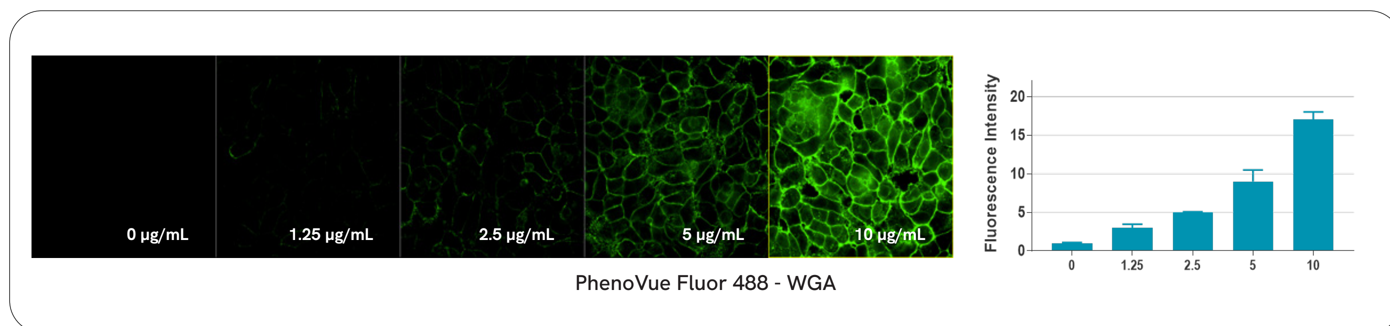


Figure 3: HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed but not permeabilized and stained with increasing concentrations of PhenoVue Fluor 488 - WGA for 10 minutes at RT. Images were acquired on the Operetta CLS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

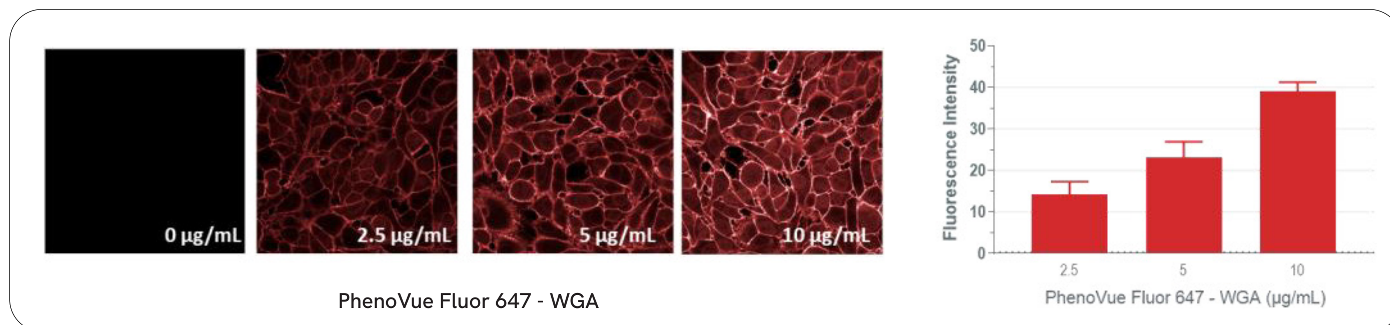
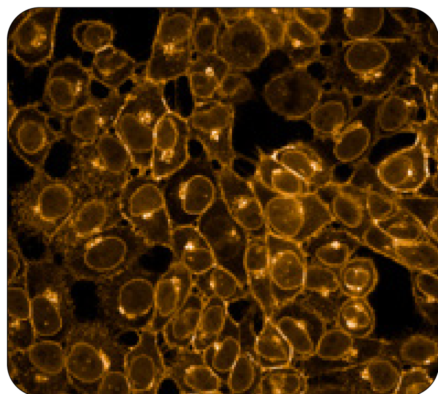
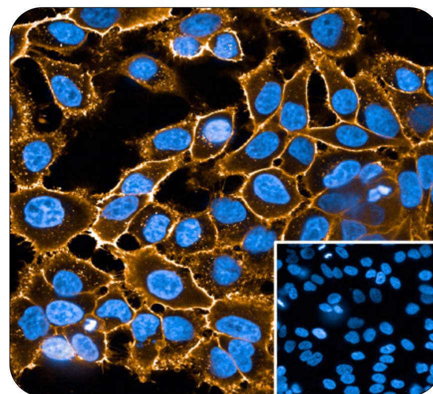


Figure 4: HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed (but not permeabilized) and stained with increasing concentrations of PhenoVue Fluor 647 - WGA for 10 min at RT. Images were acquired on the Operetta CLS high-content analysis system.



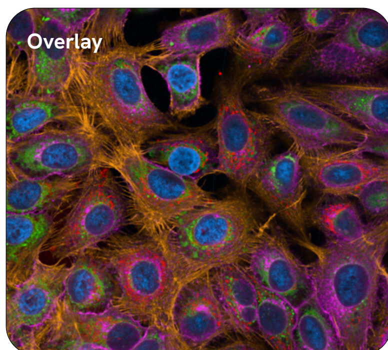
PhenoVue Fluor 555 - WGA

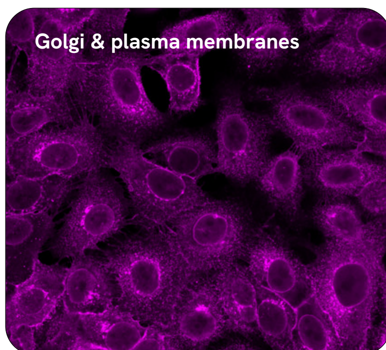
Figure 5: HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and stained with 5 µg/ mL of **PhenoVue Fluor 555 - WGA** for 10 min at RT. Images were acquired on the Operetta CLS high-content analysis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

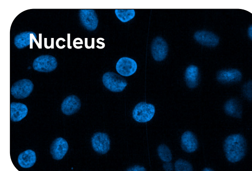


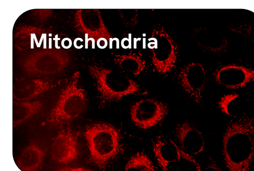
PhenoVue Fluor 568 - WGA

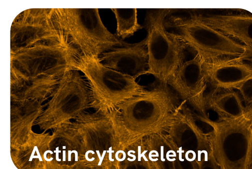
Figure 6: HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed and stained with 5 µg/mL of PhenoVue Fluor 568 - WGA for 10 min at RT. Background staining (inset negative condition) is obtained by pre-incubating non-fluorescent WGA (100X, 30min) before the PhenoVue Fluor 568 - Concanavalin A. Images were acquired on the Operetta CLS high-content analysis system.











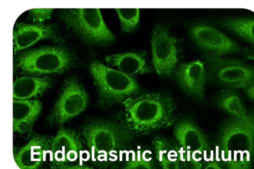


Figure 7: 5-plex experiment. HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were first stained with **PhenoVue 641 mitochondrial stain** (500 nM, Red) for 30 min at 37 °C + 5% CO_2. Cells were then fixed (PhenoVue paraformaldehyde 4%, 10 min at RT) then permeabilized (PhenoVue permeabilization 0.1% Triton X-100 solution – 10 min at RT). Cells were then stained with a mix of **PhenoVue Hoechst 33342 nuclear stain** (30 ng/mL, blue) + **PhenoVue Fluor 400LS WGA** (10 µg/mL, fuschia) + **PhenoVue Fluor 488 - Concanavalin A** (5 µg/mL, green) + **PhenoVue Fluor 568 – Phalloidin** (8nM, orange) in PhenoVue dye diluent A for 30 min at RT, and finally washed two times with HBSS. Images were acquired on the Operetta CLS (8 LED, 1600) high-content analysis system with the 63X water objective with the acquisition settings recommended in the table above.

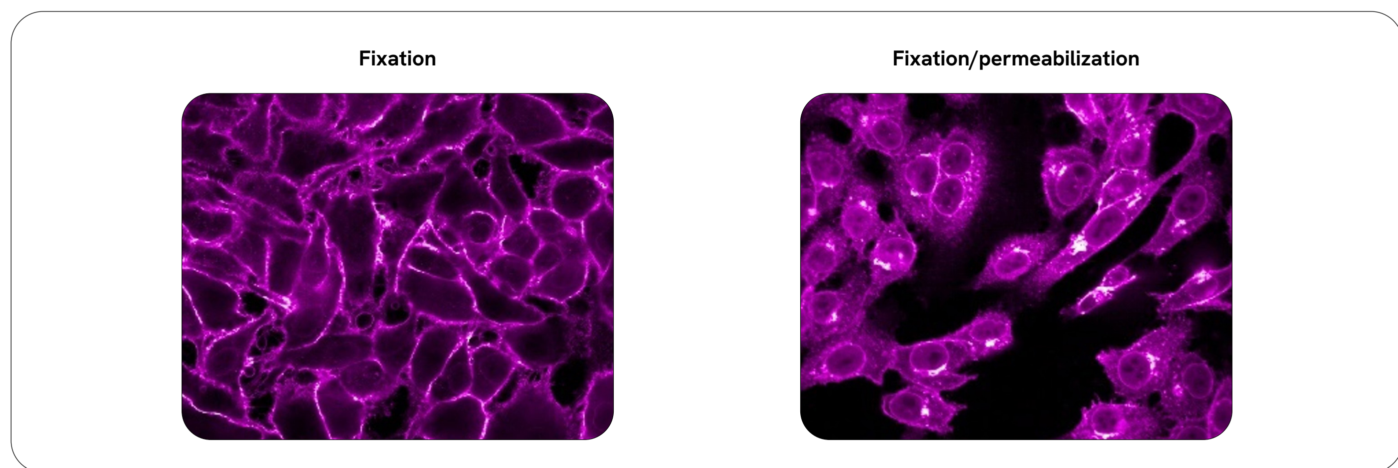
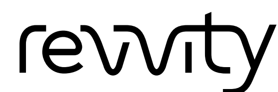


Figure 8: HeLa cells were seeded in PhenoPlate 96-well microplates (40,000 cells/well), either fixed with PhenoVue paraformaldehyde 4% solution (left panel) or fixed with PhenoVue paraformaldehyde 4% solution then permeabilized with PhenoVue permeabilization 0.1% Triton X-100 solution (right panel). Cells were stained **with PhenoVue Fluor 400LS – WGA** (30 µg/mL) in HBSS for 30 min at RT. Images were acquired on the Opera Phenix Plus (right panel) or Operetta CLS (right panel) high-content analysis system with 63X magnification and confocal mode. Fixed (but not permeabilized) cells display plasma membrane staining (left panel) while fixed and permeabilized cells display Golgi Apparatus and cytoplasmic membrane staining.

For research use only. Not for use in diagnostic procedures.



Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

Copyright ©2024, Revvity, Inc. All rights reserved.

1389171