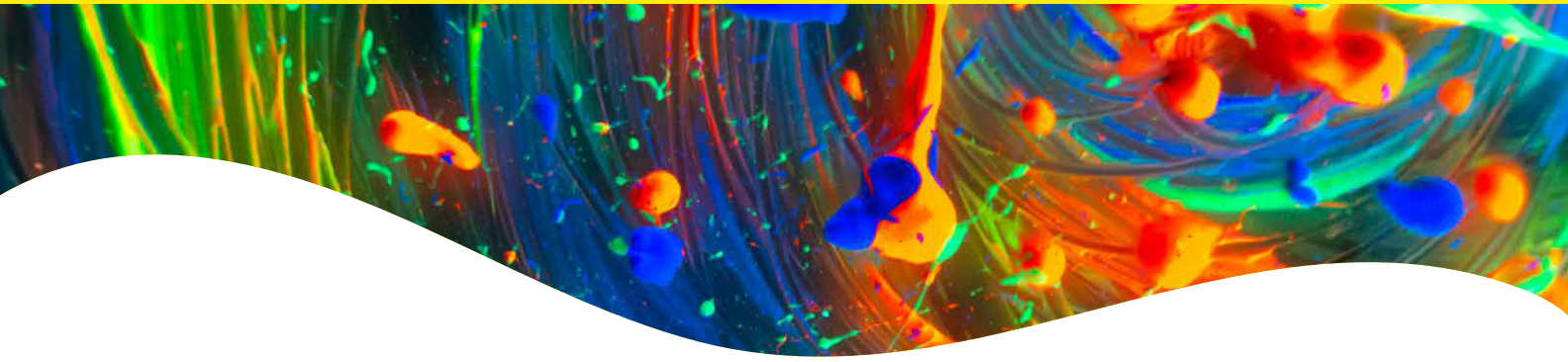
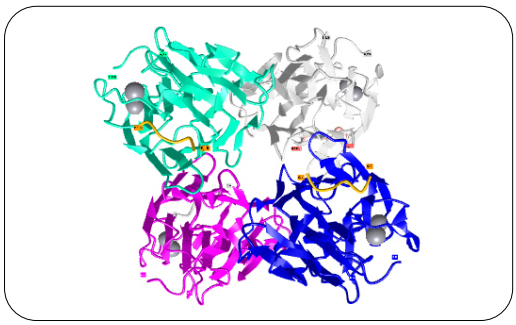
PhenoVue Fluor - Concanavalin A Conjugates



Overview

Concanavalin A is a homotetrameric lectin, also named carbohydrate binding protein, known to activate the immune system or induce apoptosis and autophagy. Concanavalin A displays high affinity for α-mannopyranosyl and α-glucopyranosyl residues of glycoproteins and glycolipids present at the cellular membranes. Therefore, fluorescent Concanavalin A conjugates represent a method of choice for labelling the cellular membranes of mammalian cells, particularly the endoplasmic reticulum which is glycoprotein enriched.

PhenoVue™ Fluor - Concanavalin A fluorescent probes can be used to visualize cellular membranes in immunofluorescence, and immunohistochemistry, as well as high-content analysis and screening applications.



Structure of Concanavalin A (Concanavalin A-hexapeptide Complex). Source: *PDB ID 1JOJ. Wang J, et al. iCn3D, a web- 1D/2D based 3D viewer for sharing /3D representations of biomolecular structures. Bioinformatics. 2020.*

Product information

Product name	Part no.	Number of vials per unit	Quantity per vial	Format	Shipping conditions
PhenoVue Fluor 488 - Concanavalin A	CP94881	5	1 mg (9.62 nmoles)	Lyophilized	RT
PhenoVue Fluor 400LS - Concanavalin A	CP94001	2	1 mg (9.62 nmoles)	Lyophilized	RT
PhenoVue Fluor 555 - Concanavalin A	CP95551	5	1 mg (9.62 nmoles)	Lyophilized	RT
PhenoVue Fluor 568 - Concanavalin A	CP95681	5	1 mg (9.62 nmoles)	Lyophilized	RT
PhenoVue Fluor 594 - Concanavalin A	CP95941	5	1 mg (9.62 nmoles)	Lyophilized	RT
PhenoVue Fluor 647 - Concanavalin A	CP96471	5	1 mg (9.62 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	Recommendedstock concentration	Working concentration range*
PhenoVue Fluor 488 - Concanavalin A	104000 g/mol	Reconstitution using 500 µL ddH_2O gives a stock concentration of 2 mg/mL (19.2 µM)	25 µg/mL – 200 µg/mL (0.24 µM – 1.92 µM)
PhenoVue Fluor 400LS - Concanavalin A	104000 g/mol	Reconstitution using 500 µL ddH_2O gives a stock concentration of 2 mg/mL (19.2 µM)	10 µg/mL – 100 µg/mL (0.096 µM – 0.96 µM)
PhenoVue Fluor 555 - Concanavalin A	104000 g/mol	Reconstitution using 500 µL ddH_2O gives a stock concentration of 2 mg/mL (19.2 µM)	25 µg/mL – 200 µg/mL (0.24 µM – 1.92 µM)
PhenoVue Fluor 568 - Concanavalin A	104000 g/mol	Reconstitution using 500 µL ddH_2O gives a stock concentration of 2 mg/mL (19.2 µM)	25 µg/mL – 200 µg/mL (0.24 µM – 1.92 µM)
PhenoVue Fluor 594 - Concanavalin A	104000 g/mol	Reconstitution using 500 µL ddH_2O gives a stock concentration of 2 mg/mL (19.2 µM)	25 µg/mL – 200 µg/mL (0.24 µM – 1.92 µM)
PhenoVue Fluor 647 - Concanavalin A	104000 g/mol	Reconstitution using 500 µL ddH_2O gives a stock concentration of 2 mg/mL (19.2 µM)	25 µg/mL – 200 µg/mL (0.24 µM – 1.92 µM)

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

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 - Concanavalin A	100 µg/mL (0.96 µM)	1 to 5	1 to 5	3 to 8
PhenoVue Fluor 400LS - Concanavalin A	50 µg/mL (0.48 µM)	1 to 4	1 to 4	2 to 6.5
PhenoVue Fluor 555 - Concanavalin A	100 µg/mL (0.96 µM)	1 to 5	1 to 5	3 to 8
PhenoVue Fluor 568 - Concanavalin A	100 µg/mL (0.96 µM)	1 to 5	1 to 5	3 to 8
PhenoVue Fluor 594 - Concanavalin A	100 µg/mL (0.96 µM)	1 to 5	1 to 5	3 to 8
PhenoVue Fluor 647 - Concanavalin A	100 µg/mL (0.96 µM)	1 to 5	1 to 5	3 to 8

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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 Concanavalin A	495	520	FITC	92%	73000	65320
PhenoVue Fluor 400LS - Concanavalin A	395	585	Ex: 375-440 Em: 550-650	nd	26000	nd
PhenoVue Fluor 555 - Concanavalin A	555	570	Cy3	10%	155000	15500
PhenoVue Fluor 568 - Concanavalin A	578	603	Texas-Red	69%	88000	60720
PhenoVue Fluor 594 - Concanavalin A	590	617	Texas-Red	66%	92000	60720
PhenoVue Fluor 647 - Concanavalin A	650	670	Cy5	30%	240000	72000

* PBS or HBSS 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 - Concanavalin A	Yes	Yes	Yes
PhenoVue Fluor 400LS - Concanavalin A	Yes	Yes	Yes
PhenoVue Fluor 555 - Concanavalin A	Yes	Yes	Yes
PhenoVue Fluor 568 - Concanavalin A	Yes	Yes	Yes
PhenoVue Fluor 594 - Concanavalin A	Yes	Yes	Yes
PhenoVue Fluor 647 - Concanavalin A	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.

Fixed-Cell Imaging

Note: PhenoVue Fluor - Concanavalin A conjugates are not cell-permeable, therefore fixed but non-permeabilized cells exhibit plasma membrane staining, whereas an additional permeabilization step enables staining of cytoplasmic membranes.

Rinse briefly in phosphate-buffered saline (PBS) then proceed with cell fixation.

1. **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:**
    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 10-200 µg/mL PhenoVue Fluor - Concanavalin A conjugates diluted in HBSS for 10-60 min at RT.Tips
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.
8 **Washing:** Wash once with PBS for 5 min.
9 **Acquire** images on an imaging device.

Live-cell imaging

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

1. Rinse briefly in HBSS.
2. Incubate with 25-200 µg/mL PhenoVue Fluor Concanavalin A 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 - Concanavalin A conjugates to prepare a working solution. It may help to reduce non-specific background.
- The homotetrameric Concanavalin A structure can bind 4 carbohydrate moieties (1 per subunit). Binding requires the presence of Mn2+ and Ca2+ ions which maintain Concanavalin A ternary structure, stability and binding activity. Avoid using buffers containing EDTA during the staining procedure.
- Concanavalin A tetrameric structure is stable at neutral pH (7-7.4). It dissociates into dimers at low pH (< 5-6) and aggregates at high pH (> 7.4). For reproducible 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 - Concanavalin A 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 endoplasmic reticulum.
- In live-cell imaging experiments, PhenoVue Fluor - Concanavalin A conjugates stain plasma membrane and eventually intracellular vesicles after invagination of the plasma membranes.

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

PhenoVue Fluor 400LS - Concanavalin A 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 - Concanavalin A 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

Validation data

PhenoVue Fluor 488 - Concanavalin A

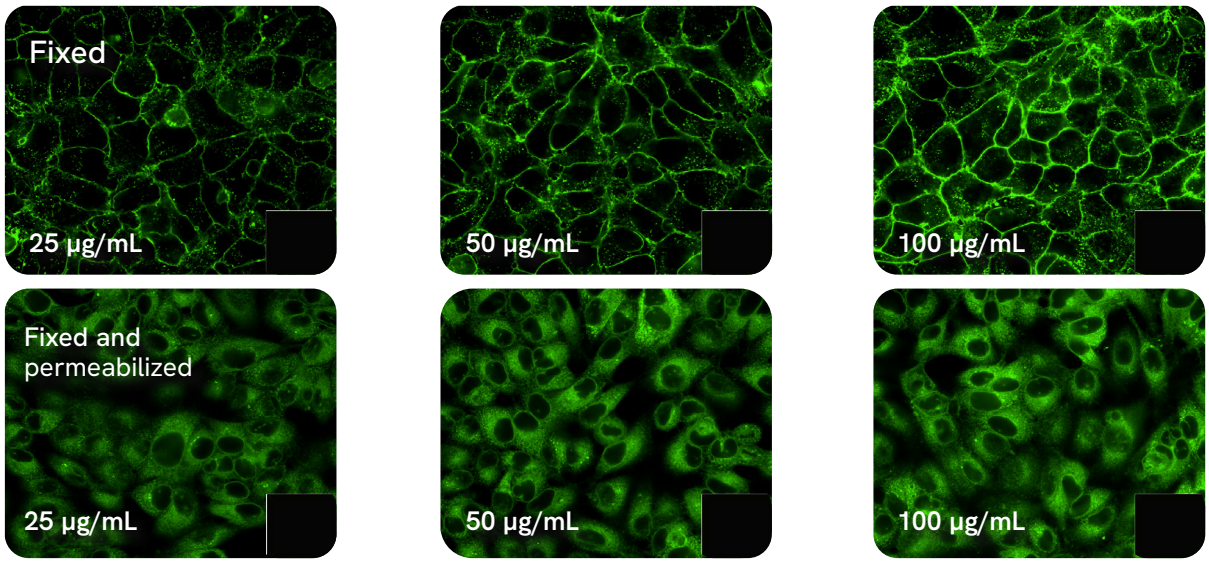


Figure 1: HeLa cells were seeded in PhenoPlate™ 96-well microplates (50,000 cells/well), either fixed or fixed then permeabilized. Cells were stained with increasing concentrations of **PhenoVue Fluor 488 - Concanavalin A** for 30 min at RT. Background staining (dark images) is obtained by pre-incubating non-fluorescent Concanavalin A (100X, 10 mg/mL/ 30 min) before the addition of the PhenoVue Fluor 488 - Concanavalin A. Images were acquired on the Operetta CLS™ high-content analysis system.

PhenoVue Fluor 555 - Concanavalin A

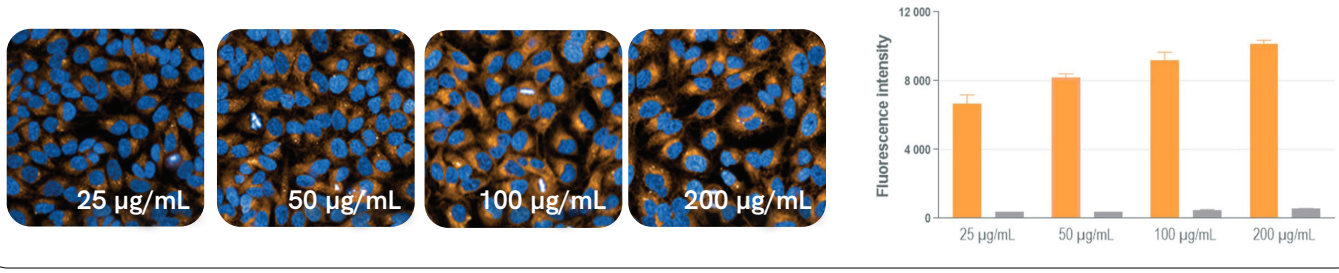
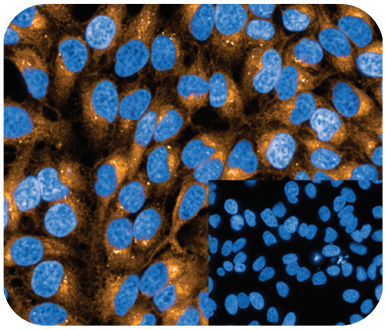


Figure 2: HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well), fixed then permeabilized. Cells were stained with increasing concentrations of **PhenoVue Fluor 555 - Concanavalin A** for 30 min at RT. Background staining (grey bars) is obtained by pre-incubating non-fluorescent Concanavalin A (100X, 10 mg/mL/30 min) before the addition of the PhenoVue Fluor 555 - Concanavalin A. Images were acquired on the Operetta CLS high-content analysis system.

PhenoVue Fluor 568 - Concanavalin A



PhenoVue Fluor 594 - Concanavalin A

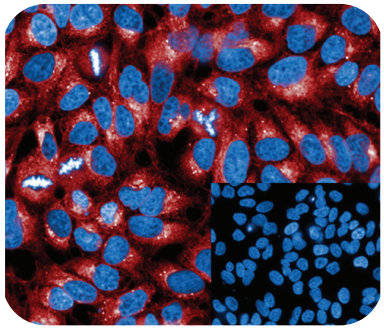
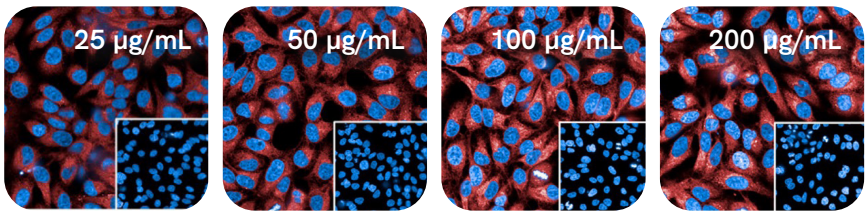


Figure 3: HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well), fixed then permeabilized. Cells were stained with 100 µg/mL of **PhenoVue Fluor 568 or 594 - Concanavalin A** for 30 min at RT. Background staining (inset image) is obtained by pre-incubating non-fluorescent Concanavalin A (100X, 10 mg/mL/30 min) before the addition of the PhenoVue Fluor 568 or 594 - Concanavalin A. Images were acquired on the Operetta CLS high-content analysis system.

PhenoVue Fluor 647 - Concanavalin A



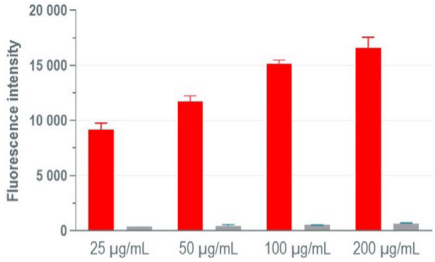


Figure 4: HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well), fixed then permeabilized. Cells were stained with increasing concentrations of **PhenoVue Fluor 647 - Concanavalin A** for 30 min at RT. Background staining (insert images, grey bars) is obtained by pre-incubating non-fluorescent Concanavalin A (100X, 10 mg/mL/30 min) before the addition of the PhenoVue Fluor 647 - Concanavalin A. Images were acquired on the Operetta CLS high-content analysis system.

PhenoVue Fluor 400LS - Concanavalin A

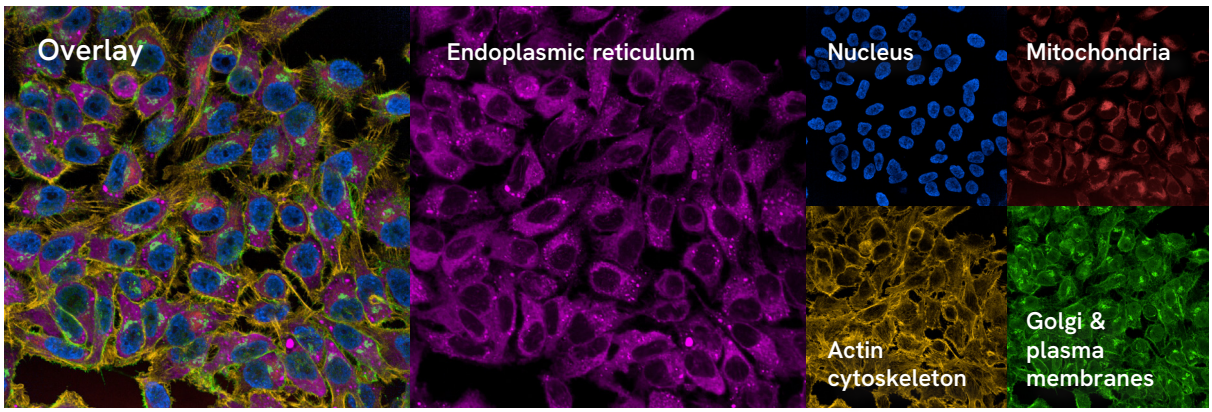


Figure 5: HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well) and incubated at 37°C, 5% CO_2 for 24h. Live cells were stained with **PhenoVue 641 mitochondrial stain** (500 nM) for 30 min at 37°C. After fixation and permeabilization, cells were stained with a solution comprising **PhenoVue Hoechst 33342** (30 ng/mL) , **PhenoVue Fluor 568 Phalloidin** (8.25 nM) , **PhenoVue Fluor 488 WGA** (10 µg/mL) and **PhenoVue Fluor 400LS Concanavalin A** (50 µg/mL) for 30 min at RT. Images were acquired on the Opera Phenix Plus 5 lasers, 63X water objective.

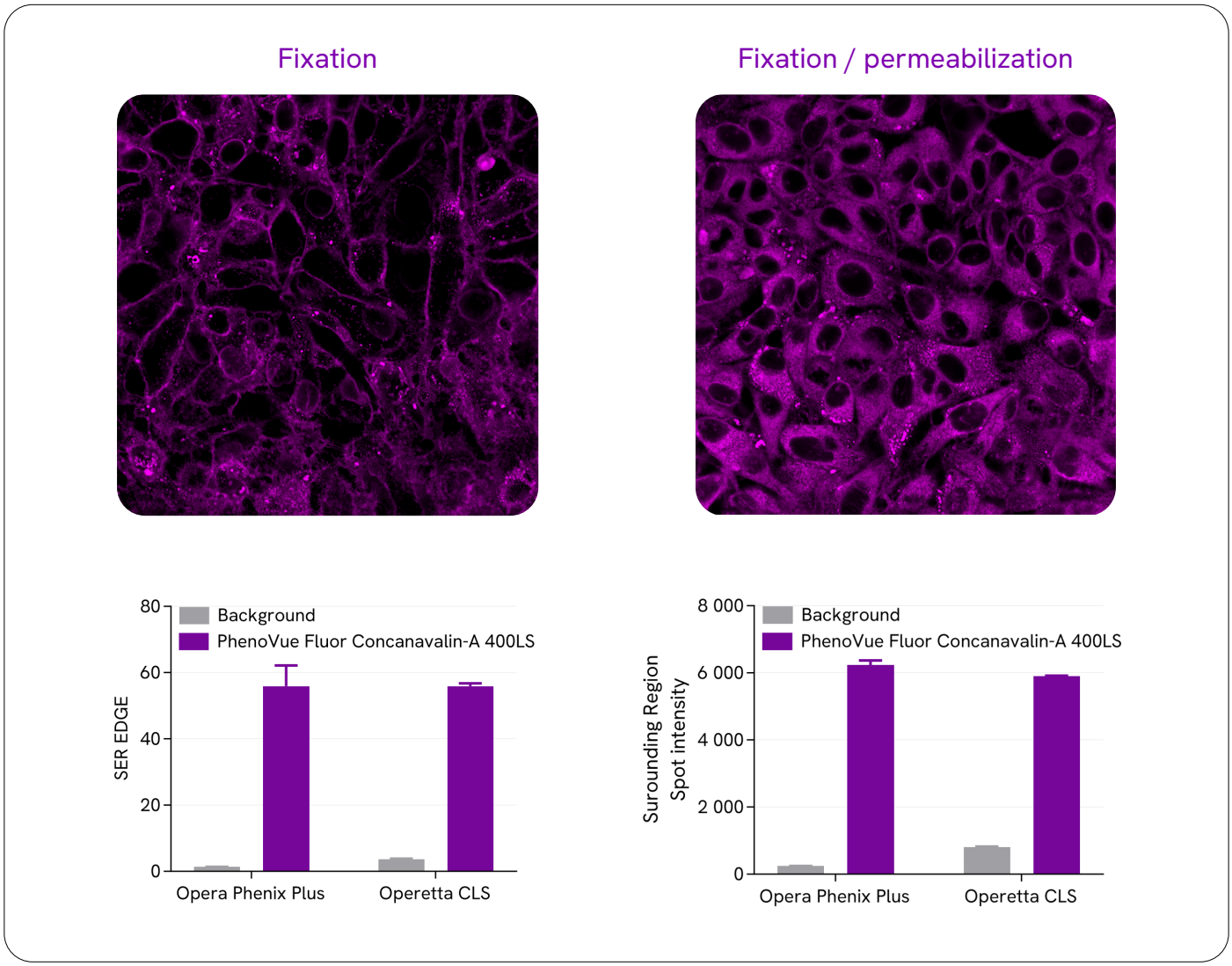


Figure 6: HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well), fixed or fixed plus permeabilized. Staining was carried out with **PhenoVue Fluor 400LS Concanavalin A** (50 µg/mL) for 30 min at RT. Images were acquired on the Opera Phenix Plus (5 lasers) and Operetta CLS (8 LED, 1600) high-content analysis system, 63X water objective.

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