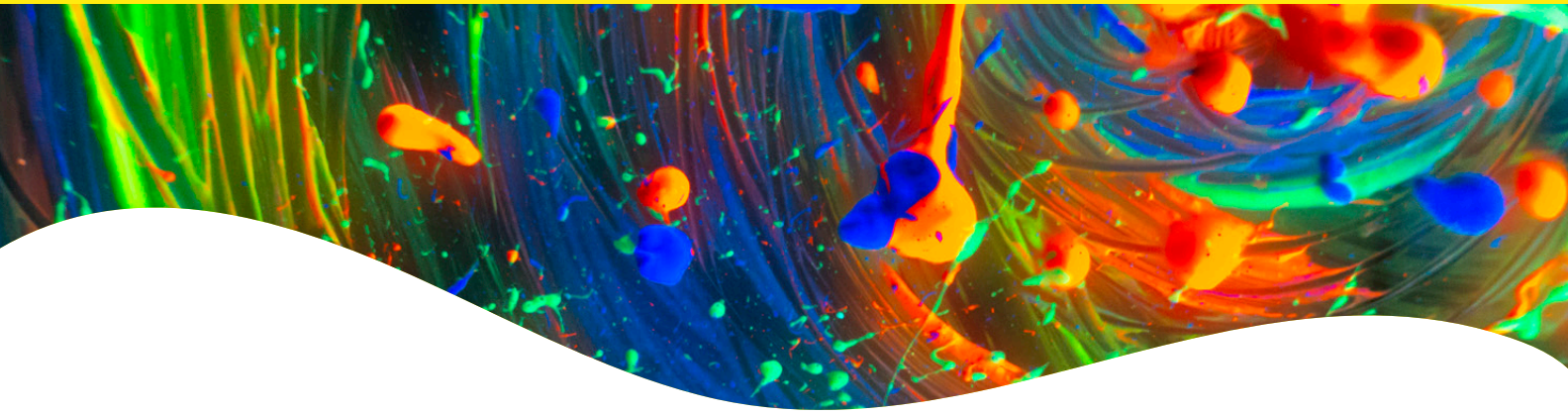
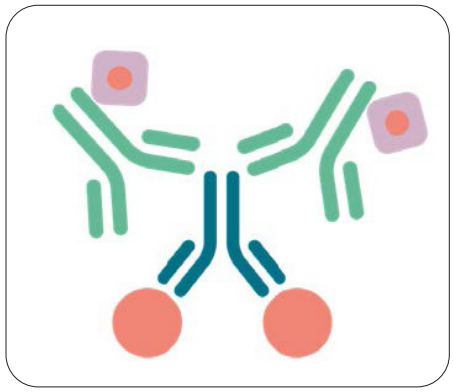
PhenoVue Fluor - Goat anti-Rat IgG (H+L) Antibody Conjugates



Overview

Goat anti-rat IgG (H+L) antibodies are conjugated with our bright PhenoVue™ Fluor dyes.

PhenoVue Fluor dyes - Goat anti-rat IgG (H+L) antibodies, highly cross-adsorbed have been adsorbed against various IgG species, such as human, rabbit, or mouse, to minimize cross-reactivity and are controlled against a broad range of other IgG species.



PhenoVue Fluor dyes

Product information

Product name	Part no.	Numbers of vials per unit	Quantity per vial	Format	Shipping conditions
PhenoVue Fluor 400LS - Goat anti-rat antibody highly cross-adsorbed	2GXRT400LSH1	1	1 mg	Lyophilized	RT
PhenoVue Fluor 405 - Goat anti-rat antibody highly cross-adsorbed	2GXRT405H1				
PhenoVue Fluor 488 - Goat anti-rat antibody highly cross-adsorbed	2GXRT488H1				
PhenoVue Fluor 555 - Goat anti-rat antibody highly cross-adsorbed	2GXRT555H1				
PhenoVue Fluor 568 - Goat anti-rat antibody highly cross-adsorbed	2GXRT568H1				
PhenoVue Fluor 594 - Goat anti-rat antibody highly cross-adsorbed	2GXRT594H1				
PhenoVue Fluor 647 - Goat anti-rat antibody highly cross-adsorbed	2GXRT647H1				

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 400LS - Goat anti-rat antibody highly cross-adsorbed	150000 g/mol	Reconstitution using 1 mL ddH_2O gives a stock concentration of 1 mg/mL (6.67 µM)	5 µg/mL – 50 µg/mL (33.3 nM – 333.3 nM)
PhenoVue Fluor 405 - Goat anti-rat antibody highly cross-adsorbed	150000 g/mol	Reconstitution using 1 mL ddH_2O gives a stock concentration of 1 mg/mL (6.67 µM)	1 µg/mL – 10 µg/mL (6.7 nM – 66.7 nM)
PhenoVue Fluor 488 - Goat anti-rat antibody highly cross-adsorbed			
PhenoVue Fluor 555 - Goat anti-rat antibody highly cross-adsorbed			
PhenoVue Fluor 568 - Goat anti-rat antibody highly cross-adsorbed			
PhenoVue Fluor 594 - Goat anti-rat antibody highly cross-adsorbed			
PhenoVue Fluor 647 - Goat anti-rat antibody highly cross-adsorbed			

* Dilutions can be done in 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 400LS - Goat anti-rat antibody highly cross-adsorbed	20 µg/mL (129.3 nM)	Approx. 1.5-5	Approx. 1.4-5	Approx. 7.5-26
PhenoVue Fluor 405 - Goat anti-rat antibody highly cross-adsorbed	5 µg/mL (32.3 nM)	Approx. 7-21	Approx. 5.5-20	Approx. 11-33
PhenoVue Fluor 488 - Goat anti-rat antibody highly cross-adsorbed	2.5 µg/mL (16.7 nM)	Approx. 14-42	Approx. 12-42	Approx. 22-65
PhenoVue Fluor 555 - Goat anti-rat antibody highly cross-adsorbed				
PhenoVue Fluor 568 - Goat anti-rat antibody highly cross-adsorbed				
PhenoVue Fluor 594 - Goat anti-rat antibody highly cross-adsorbed				
PhenoVue Fluor 647 - Goat anti-rat antibody highly cross-adsorbed				

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 filter set	Quantum yield (ϕ)	Epsilon* (ε in $M^{-1}.cm^{-1}$ at λ max)	Brightness (ϕ x ε)
PhenoVue Fluor 400LS	395	585	Ex: 375-440 nm Em: 550-650 nm	nd*	26000	Nd*
PhenoVue Fluor 405	410	452	Ex: 375-430 nm Em: 440-500 nm	78%	46000	35880
PhenoVue Fluor 488	495	520	FITC	92%	73000	65320
PhenoVue Fluor 555	555	570	Cy3	10%	155000	15500
PhenoVue Fluor 568	578	603	Texas-Red	69%	88000	60720
PhenoVue Fluor 594	590	613	Texas-Red	66%	92000	60720
PhenoVue Fluor 647	650	670	Cy5	30%	240000	72000

* Not determined

Recommended acquisition settings for Revvity HCS instruments

HCS Instruments		PhenoVue Fluor 405	PhenoVue Fluor 400LS	PhenoVue Fluor 488	PhenoVue Fluor 555	PhenoVue Fluor 568 Or PhenoVue Fluor 594	PhenoVue Fluor 647
Opera Phenix Plus 5 lasers	Excitation laser (nm)	425	425	488	561	561	640
	Emission filter (nm)	435-480	570-630	500-550	570-630	570-630	650-760
Opera Phenix Plus 4 lasers	Excitation laser (nm)	405	405	488	561	561	640
	Emission filter (nm)	435-480	570-630	500-550	570-630	570-630	650-760
Operetta CLS 8 LED - 1600	Excitation LED (filt'er) (nm)	405 (390-420)	405 (390-420)	475 (460-490)	550 (530-560)	550 (530-560)	630 (615-645)
	Emission filter (nm)	430-500	570-650	500-550	570-650	570-650	655-760
Operetta CLS 8 LED - 1601	Excitation LED (filt'er) (nm)	370 (355-385)	440 (435-460)	475 (460-490)	550 (530-560)	580 (560-575)	630 (615-645)
	Emission filter (nm)	430-500	600-640 or 570-650	500-550	570-650	585-640	655-760
Operetta CLS 4 LED	Excitation LED (filt'er) (nm)	370 (355-385)	370 (355-385)	475 (460-490)	550 (530-560)	550 (530-560)	630 (615-645)
	Emission filt'er	430-500	570-650	500-550	570-650	570-650	655-760

Cross-reactivity

Product name	Across species	Across IgG isotypes
PhenoVue Fluor 400LS - Goat anti-rat antibody highly cross-adsorbed	Rat (100%) Hamster (30%)	Cross-reactivity with rat: IgG1 IgG2a IgG2b IgG2c
PhenoVue Fluor 405 - Goat anti-rat antibody highly cross-adsorbed		
PhenoVue Fluor 488 - Goat anti-rat antibody highly cross-adsorbed		
PhenoVue Fluor 555 - Goat anti-rat antibody highly cross-adsorbed		
PhenoVue Fluor 647 - Goat anti-rat antibody highly cross-adsorbed		
PhenoVue Fluor 568 - Goat anti-rat antibody highly cross-adsorbed		
PhenoVue Fluor 594 - Goat anti-rat antibody highly cross-adsorbed		

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

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 at least 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 at least 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. Blocking step: Incubate with PBS + 1% BSA for 60 min at RT.

6. Primary antibody: Incubate with a primary rat antibody.

7. Washing: Wash three times with PBS for 5 min.

8. Staining: Incubate with 1-10 µg/mL PhenoVue Fluor-Goat anti-rat antibody highly cross-adsorbed for 60 min at RT. (5 – 50 µg/mL of PhenoVue Fluor 400LS Goat anti-mouse antibody highly cross-adsorbed)

Note that use of higher concentrations and a longer incubation could significantly improve signal over background, particularly for PhenoVue Fluor 400LS conjugates.

9. Washing: Wash three times with PBS for 5 min.

10. **Optional:** Incubate with 0.02-2 µg/mL PhenoVue Hoechst 33342 nuclear stain for 10 min or 2-10 µM PhenoVue DRAQ5 Total Cell Nuclear Stain for 15-60 min.

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

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

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

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

Although PhenoVue Fluor 400LS dye is one of the brightest long Stokes' shift dyes, it is less bright than standard PhenoVue Fluor dyes. It requires higher concentrations and longer and/or increased laser power excitation especially if the target expression is low.

To obtain the highest 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.
  - With 365 nm excitation (e.g Operetta CLS - 4 LED), please note that crosstalk of Hoechst 33342 (or DAPI) is inevitable.
- Excitation of PhenoVue Fluor 400LS with greater than 415 nm (e.g. Operetta CLS with 440 nm excitation):
  - In this configuration and in the case of multiplexing with a PhenoVue Fluor 488 probe, concentrations might need to be adjusted to limit the crosstalk of PhenoVue Fluor 488 in the PhenoVue Fluor 400LS channel. Note that the 600-640 nm emission band can also be used for PhenoVue Fluor 400LS to limit this crosstalk, but PhenoVue Fluor 400LS intensity staining will also be reduced.
- With the Operetta CLS – 4 LED system, please note that use of PhenoVue Fluor 400LS conjugates in the presence of Hoechst (or DAPI) is not recommended
- For simultaneous acquisition (e.g. Opera Phenix/Plus):
  - Separate the 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 conjugates may result in an emission in the 570-630 nm detection band.

Tips

- Use PhenoVue Fluor - Goat anti-rat highly cross-adsorbed antibodies when performing multiplexing experiments including different primary antibodies (see Figures 4, 5 and 6). Please note that this is not limited to PhenoVue secondary antibodies but rather a general characteristic of antibodies, irrespective of the vendor.

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
- Flow cytometry

Validation data

PhenoVue Fluor 488 - Goat anti-rat IgG (H+L) highly cross-adsorbed

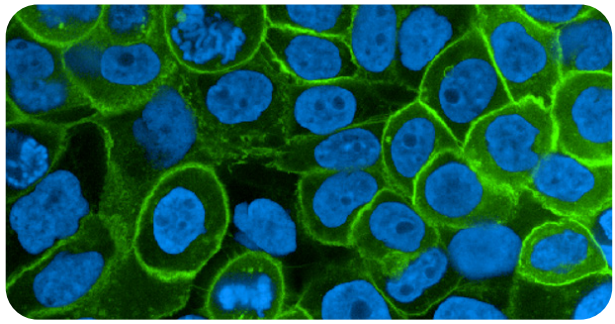
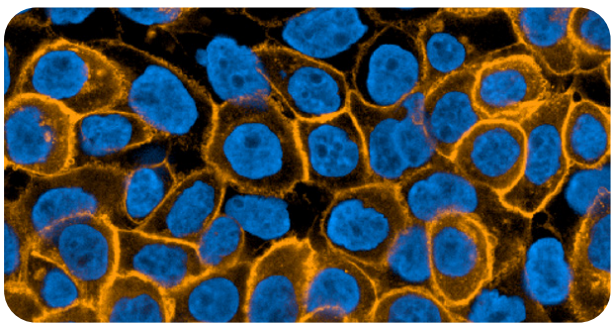


Figure 1: A431 cells were seeded in PhenoPlate™ 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and incubated with an anti-EGFR rat IgG2a antibody (2 µg/mL). After washing steps, cells were incubated with 10 µg/mL of PhenoVue Fluor 488 - Goat anti-rat IgG (H+L) highly cross-adsorbed for 1 hour at RT. Nuclei were stained with 2 µg/mL PhenoVue Hoechst 33342 nuclear stain. Images were acquired on the Operetta CLS™ high-content analysis system, using 63x water objective, confocal mode.

A PhenoVue Fluor 555 - Goat anti-rat IgG (H+L) highly cross-adsorbed



B PhenoVue Fluor 647 - Goat anti-rat IgG (H+L) highly cross-adsorbed

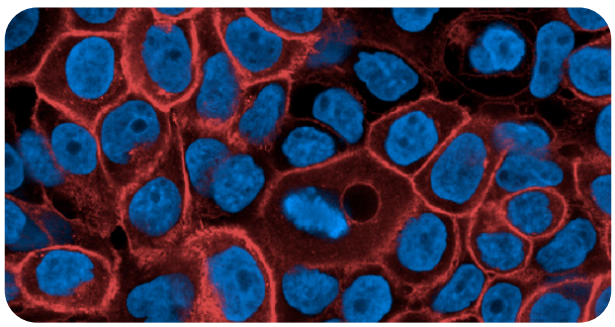


Figure 2: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and incubated with an anti-EGFR rat IgG2a antibody (2 µg/mL). After washing steps, cells were incubated with 10 µg/mL of PhenoVue Fluor 555 - Goat anti-rat highly cross-adsorbed (A) or PhenoVue Fluor 647 - Goat anti-rat highly cross-adsorbed (B) for 1 hour at RT. Nuclei were stained with 2 µg/mL PhenoVue Hoechst 33342 nuclear stain. Images were acquired on the Operetta CLS high-content analysis system using 63x water objective, confocal mode.

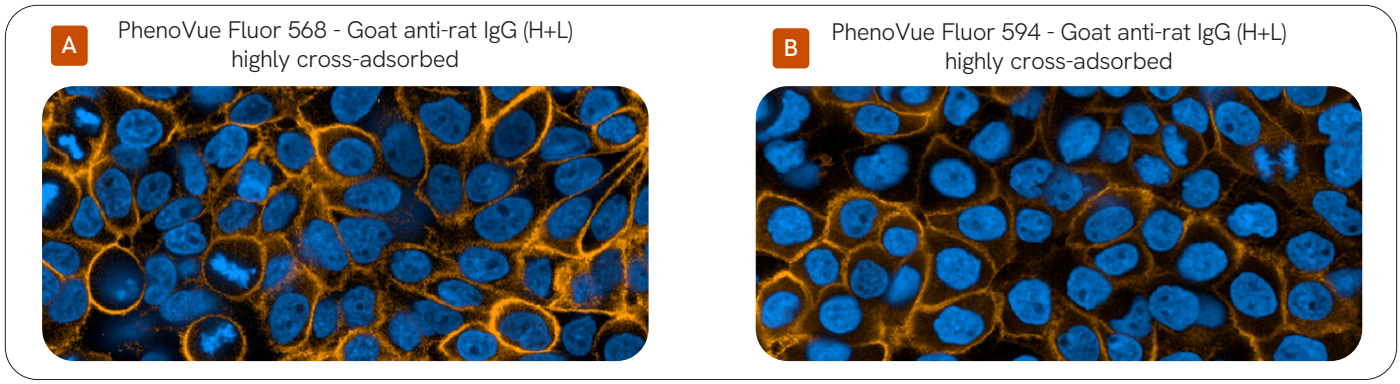


Figure 3: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and incubated with an anti-EGFR rat IgG2a antibody (2 µg/mL). After washing steps, cells were incubated with 10 µg/mL of PhenoVue Fluor 568 - Goat anti-rat highly cross-adsorbed (A) or PhenoVue Fluor 594 - Goat anti-rat highly cross-adsorbed (B) for 1 hour at RT. Nuclei were stained with 2 µg/mL PhenoVue Hoechst 33342 nuclear stain. Images were acquired on the Operetta CLS high-content analysis system using 63x water objective, confocal mode.

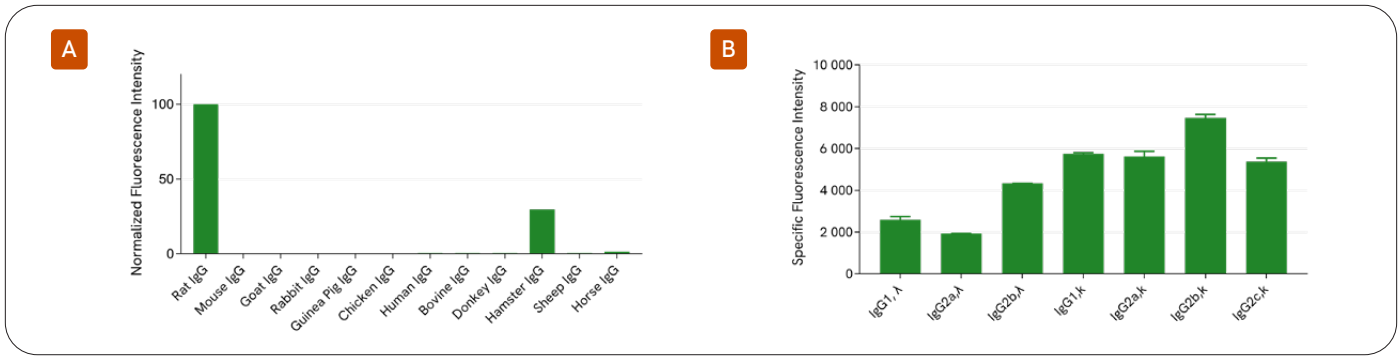


Figure 4: F-LISA experiments: different IgG species (A) or rat IgG isotypes (B) were used to coat a 96-well microplate, then incubated with PhenoVue Fluor 647 - Goat anti-rat IgG (H+L) highly cross-adsorbed (5 µg/mL). Fluorescence intensity was measured on an EnVision multimode plate reader.

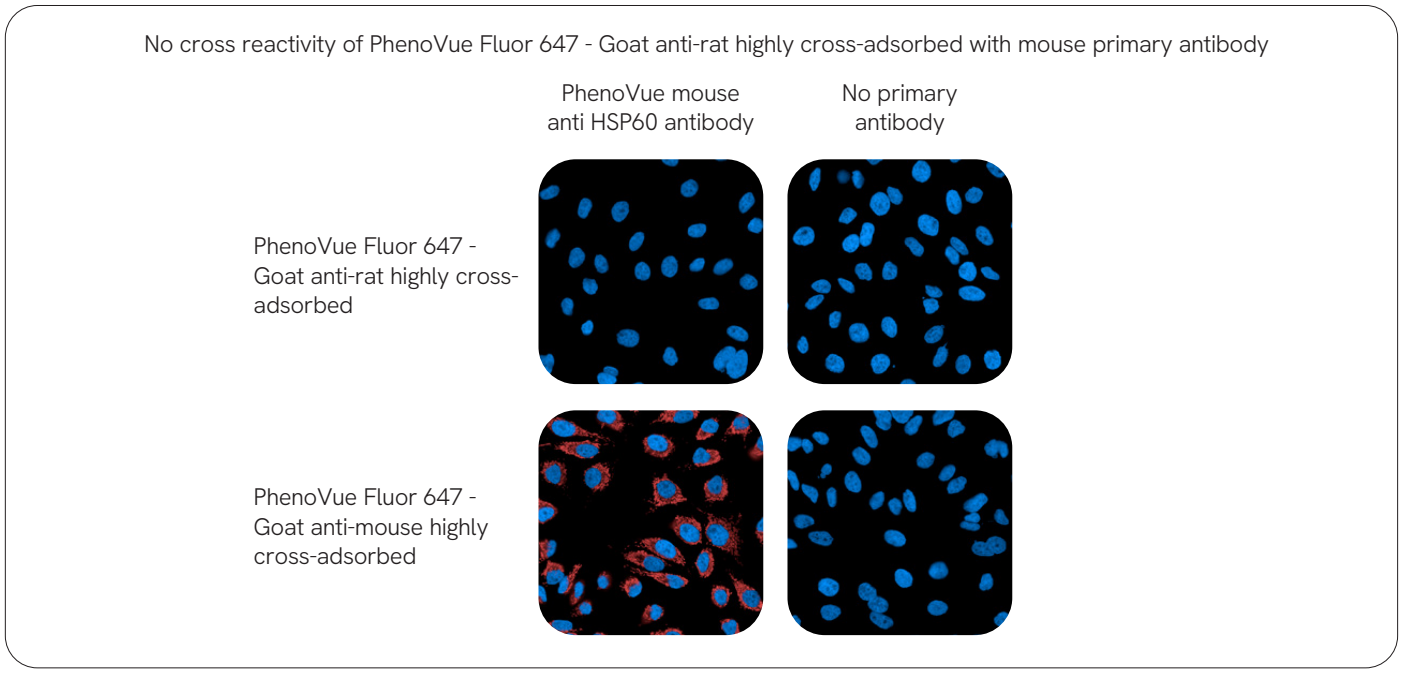


Figure 5: Hela cells were seeded in PhenoPlate 96-well microplates (35,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and incubated with PhenoVue anti-HSP60 mouse antibody (1X) over night at 4 °C. After washing steps, cells were incubated with PhenoVue Hoechst 33342 and PhenoVue Fluor 647 - Goat anti-rat IgG (H+L) highly cross-adsorbed or PhenoVue Fluor 647 – Goat anti-mouse highly cross-adsorbed antibody for 1h at RT. Images were acquired on an Operetta CLS 1800 high-content analysis system using 63x water objective, confocal mode.

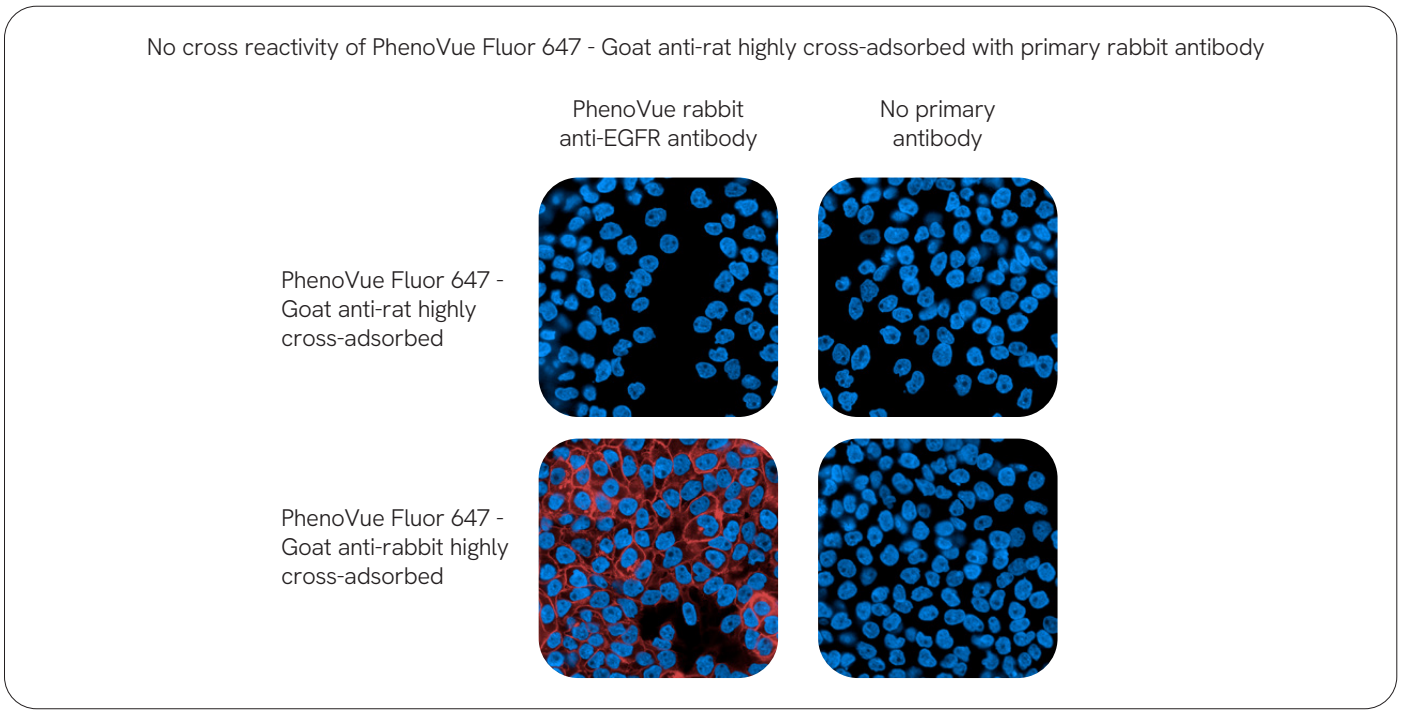


Figure 6: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and incubated with anti EGFR rabbit antibody (2µg/mL) (Panel B) 3h at RT. After washing steps, cells were incubated with PhenoVue Hoechst 33342 and PhenoVue Fluor 647 - Goat anti-rat IgG (H+L) highly cross-adsorbed or PhenoVue Fluor 647 – Goat anti-rabbit highly cross-adsorbed antibody for 1h at RT (5 µg/mL). Images were acquired on an Operetta CLS 1800 high-content analysis system using 63x water objective, confocal mode.

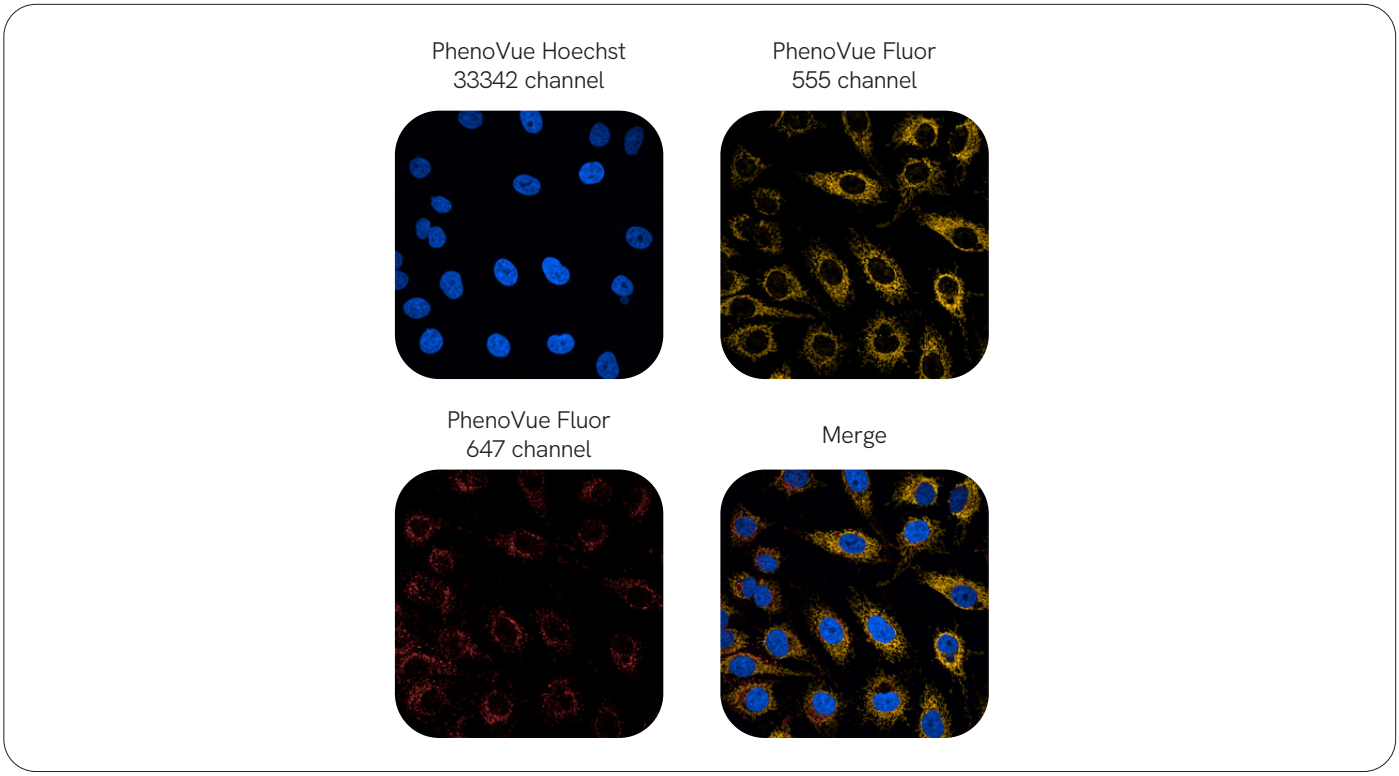


Figure 7: Hela cells were seeded in PhenoPlate 96-well microplates (35,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and incubated with PhenoVue anti- HSP60 mouse antibody (1X) and PhenoVue anti-LAMP1 IgG2bκ rat antibody (1X) over night at 4 °C. After washing steps, cells were incubated for 1h with PhenoVue Hoechst 33342, PhenoVue Fluor 647 - Goat anti-rat IgG (H+L) highly cross-adsorbed and PhenoVue Fluor 555 – Goat anti-mouse highly cross-adsorbed antibody for 1h at RT. Images were acquired on an Opera Phenix Plus HCS system using 63x water objective, confocal mode.

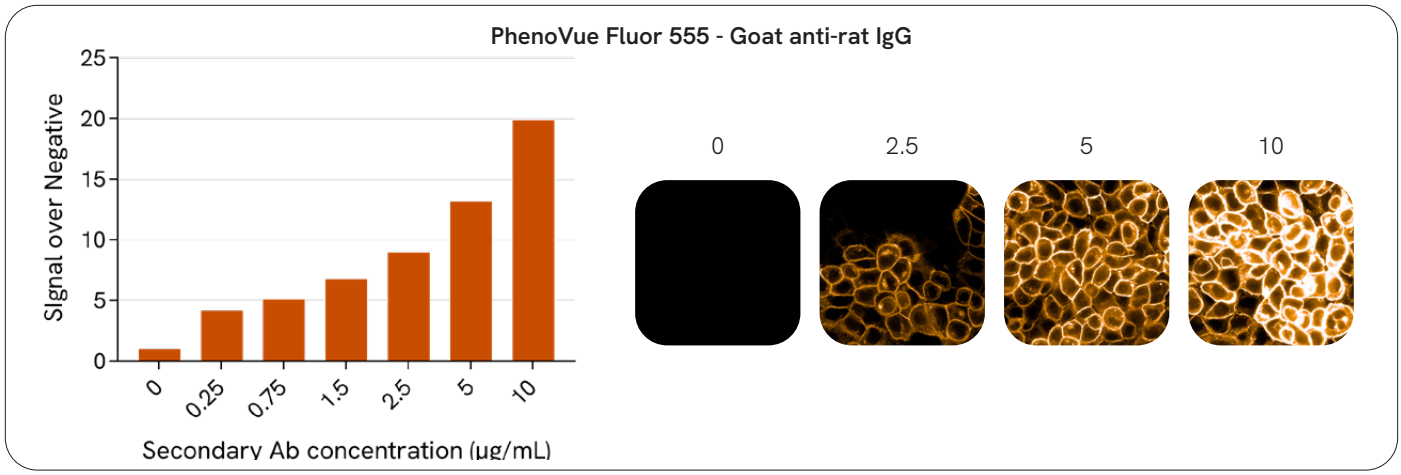


Figure 8: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and incubated with anti EGFR rat IgG2a antibody (2 µg/mL) (Panel B) 3h at RT. After washing steps, cells were incubated with PhenoVue Hoechst 33342 and PhenoVue Fluor 555-Goat anti-rat IgG (H+L) highly cross-adsorbed antibody for 1h at RT (5 µg/mL). Images were acquired on an Operetta CLS 1600 high-content analysis system using 63x water objective, confocal mode.

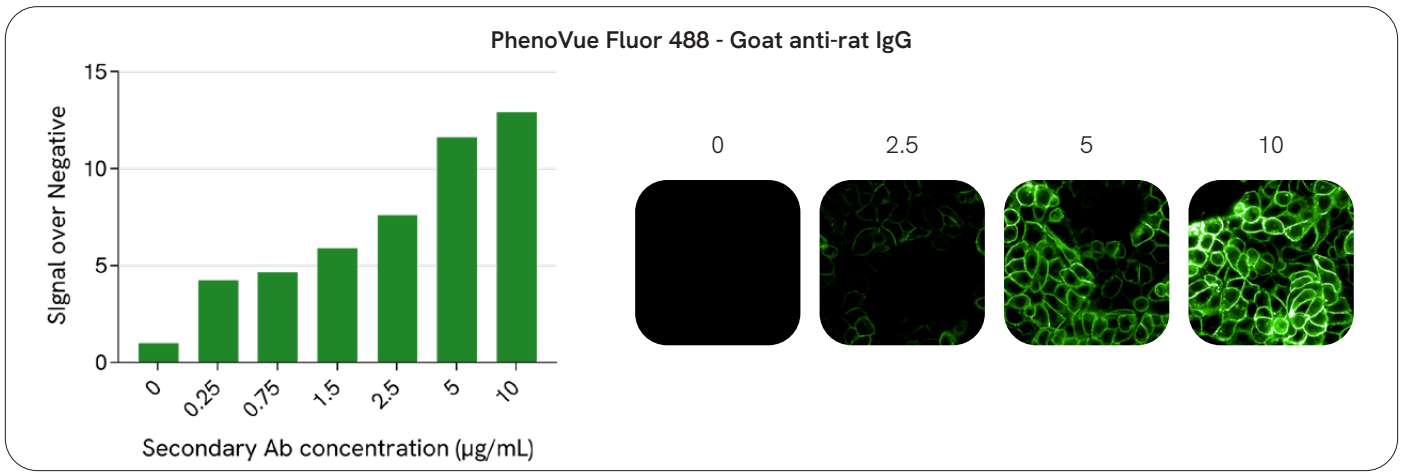


Figure 9: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and incubated with anti EGFR rat IgG2a antibody (2 µg/mL) (Panel B) 3h at RT. After washing steps, cells were incubated with PhenoVue Hoechst 33342 and PhenoVue Fluor 488-goat anti-rat IgG (H+L) highly cross-adsorbed antibodyfor 1h at RT (5 µg/mL). Images were acquired on an Operetta CLS 1800 high-content analysis system using 63x water objective, confocal mode.

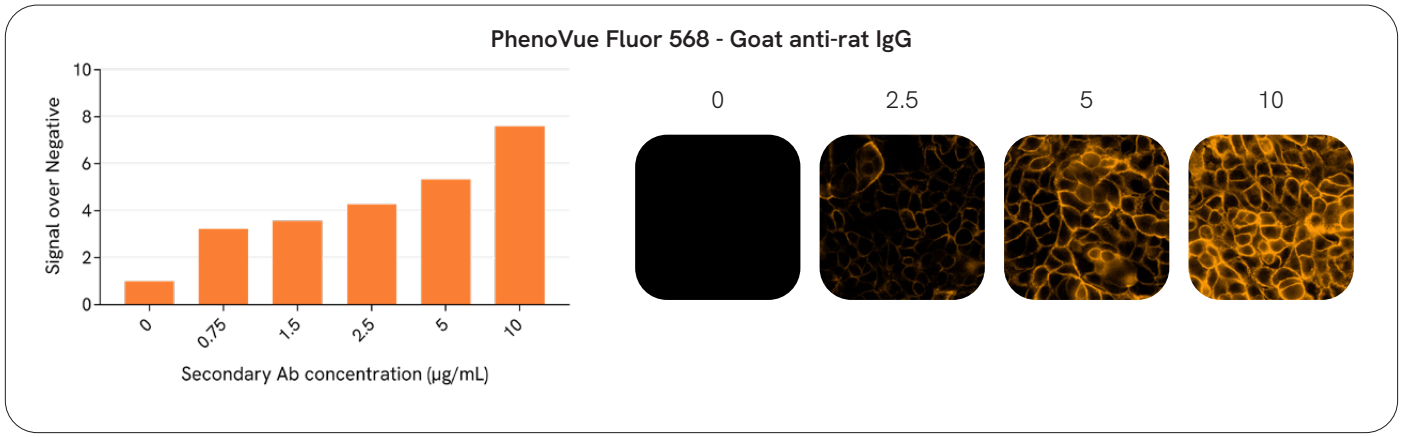


Figure 10: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and incubated with anti EGFR rat IgG2a antibody (2 µg/mL) (Panel B) 3h at RT. After washing steps, cells were incubated with PhenoVue Hoechst 33342 and PhenoVue Fluor 568-goat anti-rat IgG (H+L) highly cross-adsorbed antibody for 1h at RT. Images were acquired on an Operetta CLS 1801 high-content analysis system using 63x water objective, confocal mode.

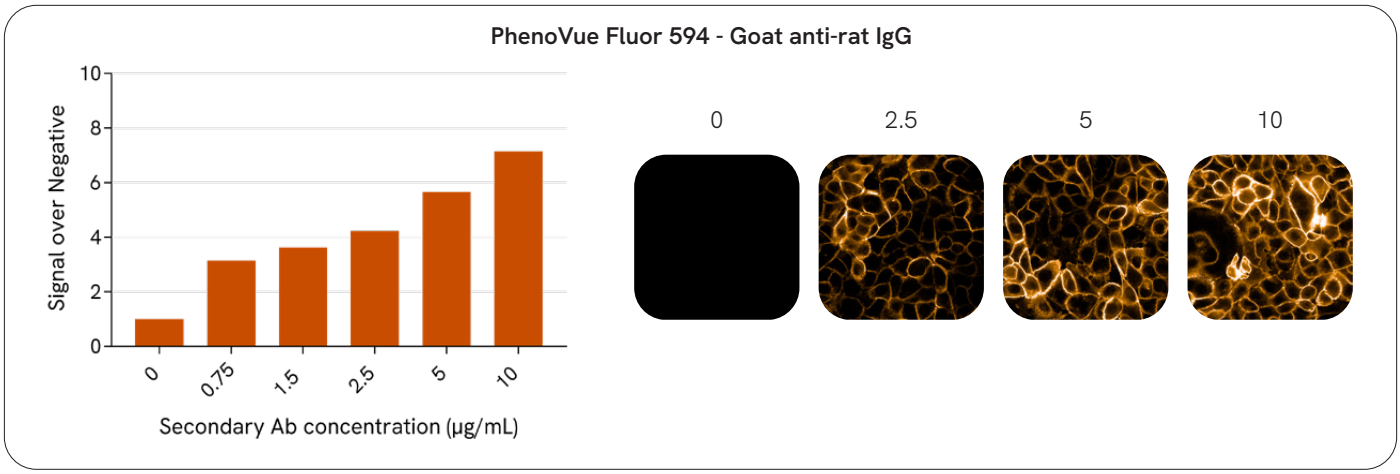


Figure 11: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and incubated with anti EGFR rat IgG2a antibody (2 µg/mL) (Panel B) 3h at RT. After washing steps, cells were incubated with PhenoVue Hoechst 33342 and PhenoVue Fluor 594-goat anti-rat IgG (H+L) highly cross-adsorbed antibody for 1h at RT. Images were acquired on an Operetta CLS 1801 high-content analysis system using 63x water objective, confocal mode.

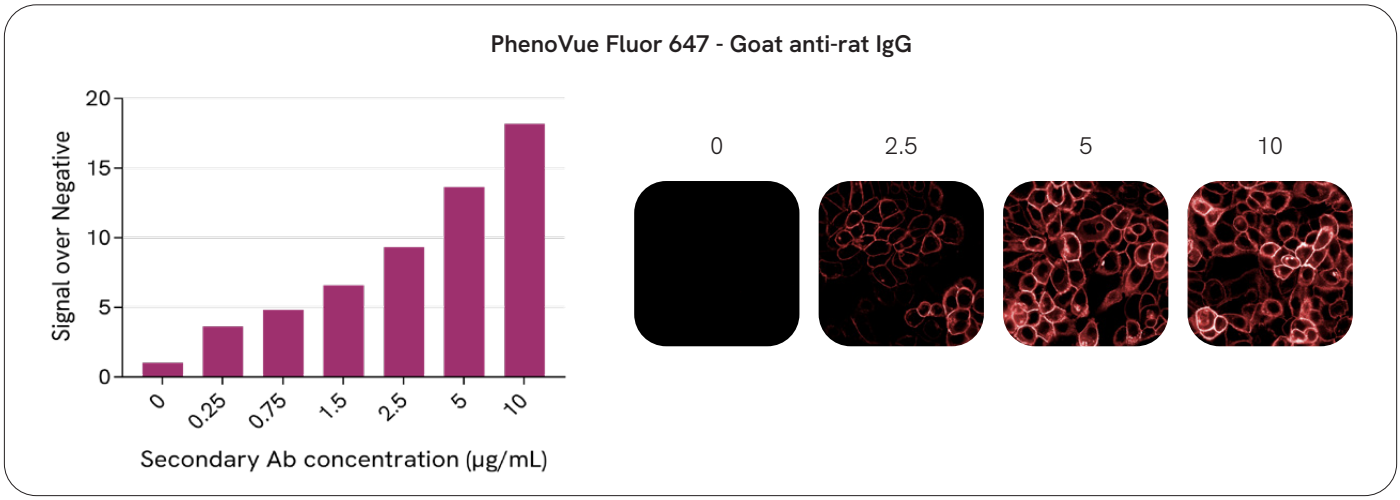


Figure 12: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and incubated with anti EGFR rat IgG2a antibody (2 µg/mL) (Panel B) 3h at RT. After washing steps, cells were incubated with PhenoVue Hoechst 33342 and PhenoVue Fluor 647-goat anti-rat IgG (H+L) highly cross-adsorbed antibody for 1h at RT. Images were acquired on an Operetta CLS 1800 high-content analysis system using 63x water objective, confocal mode.

PhenoVue Fluor 405 - Goat anti-rat IgG (H+L) highly cross-adsorbed

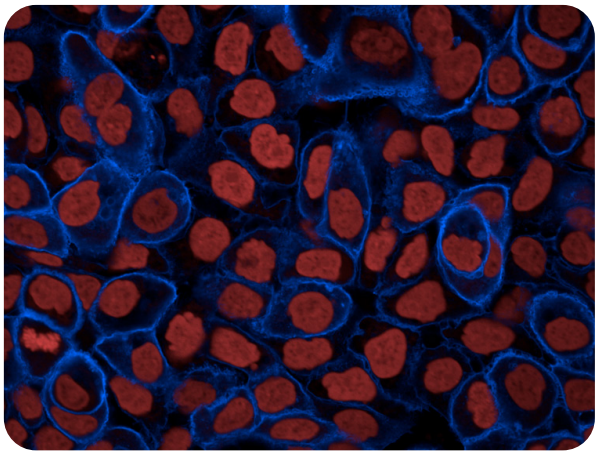


Figure 13: A431 cells were seeded in PhenoPlate™ 96-well microplates at a density of 75,000 cells per well and incubated for 24 hours at 37°C with 5% CO_2. Following incubation, the cells were fixed, permeabilized, and treated with 2 µg/mL of anti-EGFR rat IgG2a antibody. After washing, the cells were incubated with 10 µg/mL of PhenoVue Fluor 405 - Goat anti-Rat IgG (H+L), highly cross-adsorbed, for 1 hour at room temperature. The nuclei were stained with 1 µM PhenoVue DRAQ5 Total Cell Nuclear Stain. Images were acquired using the Operetta CLS 1600 high-content analysis system with a 63x water objective in confocal mode.

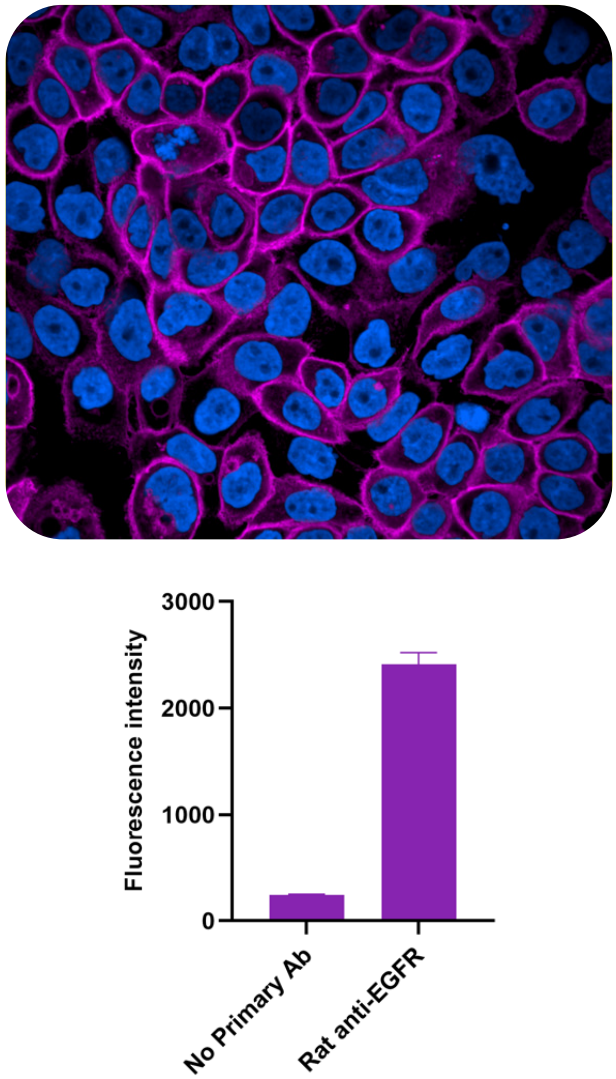


Figure 14: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and incubated with an anti-EGFR mouse antibody (2 µg/mL). After washing steps, cells were incubated with 20 µg/mL of PhenoVue Fluor 400LS - Goat anti-rat IgG (H+L) highly cross-adsorbed for 1 hour at RT. Nuclei were stained with 75 ng/mL PhenoVue Hoechst 33342 nuclear stain. Images were acquired on the Opera Phenix Plus (5 lasers) high-content imaging system using 63x water objective, confocal mode.

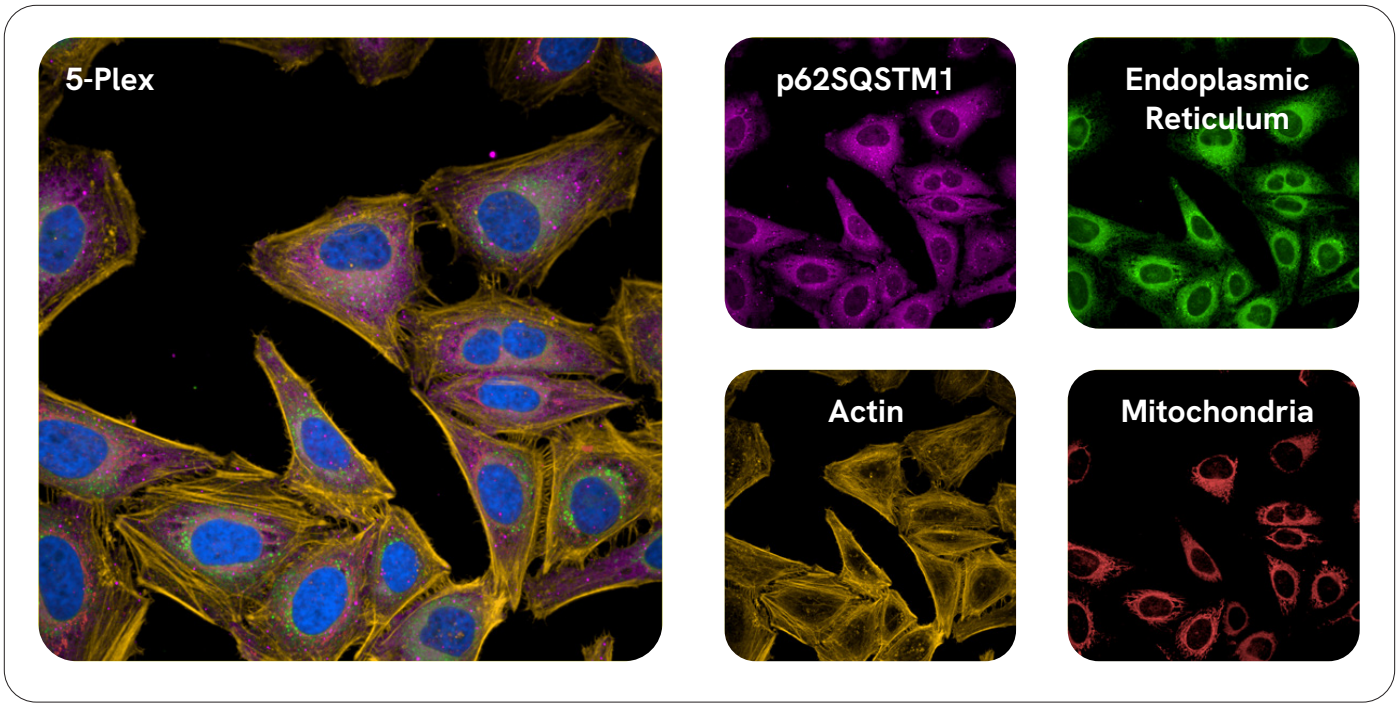


Figure 15: U2OS cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Live cells were first stained for 30 minutes with 500nM of PhenoVue 641 mitochondrial stain before fixation, permeabilization and saturation steps. Cells were then incubated overnight with rabbit anti-p62SQSTM1 (5µg/mL). After washing steps, a staining mix with 20 µg/mL of PhenoVue Fluor 400LS - Goat anti-rabbit IgG (H+L) highly cross-adsorbed, 50ng/mL of PhenoVue Hoechst 33342, 5µg/mL of PhenoVue Fluor 488 - Concanavalin A and 8nM PhenoVue Fluor 568 - Phalloidin were then added for 1 hour at RT. Images were acquired on the Opera Phenix Plus (5 lasers) high-content imaging system using 63x water objective, confocal mode.