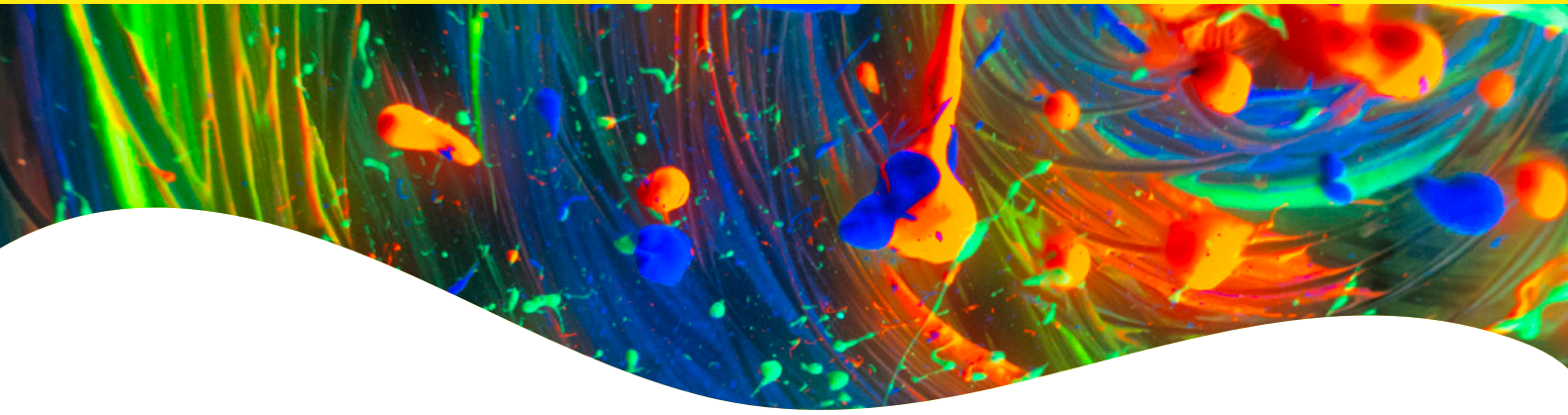
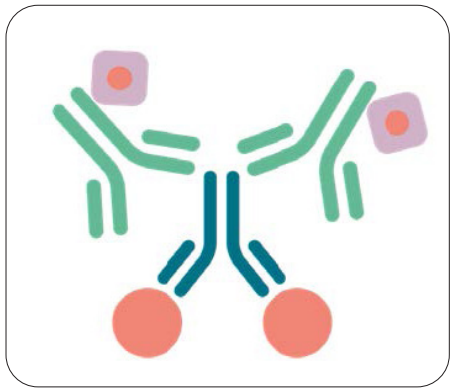
# PhenoVue Fluor - Goat anti-Rat IgG (H+L) Antibody Conjugates



## 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 488 - Goat anti-rat antibody highly cross-adsorbed | 2GXRT488H1 | 1 | 1 mg | Lyophilized | RT |
| 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 488 - Goat anti-rat antibody highly cross-adsorbed | 150000 g/mol | Reconstitution using 1 mL $ddH_2O$ gives a stock concentration of 1 mg/mL (6.66 µM) | 1 µg/mL – 10 µg/mL (6.6 nM – 66.6 nM) |
| 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 488 - Goat anti-rat antibody highly cross-adsorbed | 2.5 µg/mL (16.6 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 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 |

* In methanol

## Cross-reactivity

| Product name | Across species | Across IgG isotypes |
|---|---|---|
| PhenoVue Fluor 488 - Goat anti-rat antibody highly cross-adsorbed | Rat (100%)<br>Hamster (30%) | Cross-reactivity with rat:<br>IgG1<br>IgG2a<br>IgG2b<br>IgG2c |
| 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 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. 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.

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

**10. Optional:** Incubate with 0.1-2 µg/mL PhenoVue Hoechst 33342 nuclear stain for 10 min.

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

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

## 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

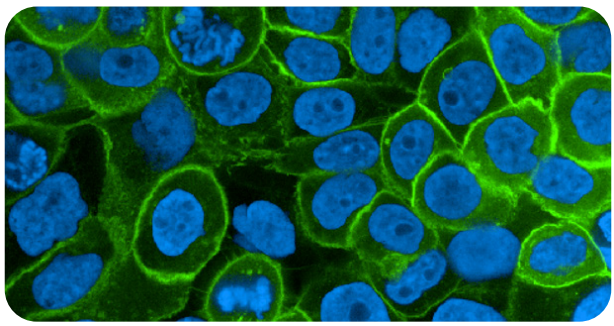
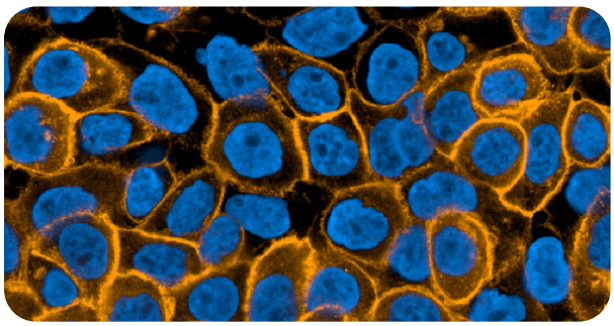


Figure 1: A431 cells were seeded in PhenoPlate™ 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% $CO_2$ for 24h. Cells were fixed then permeabilized and incubated with an anti-EGFR rat IgG2a antibody (2 µg/mL). After washing steps, cells were incubated with 10 µg/mL of PhenoVue Fluor 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

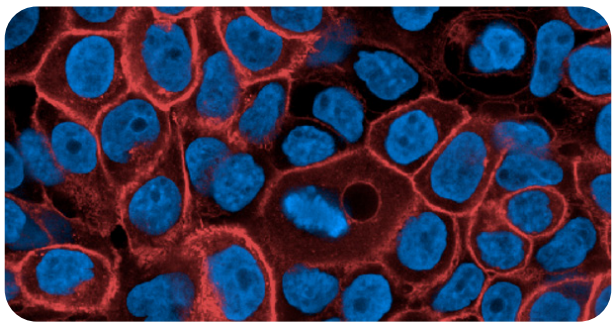


Figure 2: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% $CO_2$ for 24h. Cells were fixed then permeabilized and incubated with an anti-EGFR rat IgG2a antibody (2 µg/mL). After washing steps, cells were incubated with 10 µg/mL of PhenoVue Fluor 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.

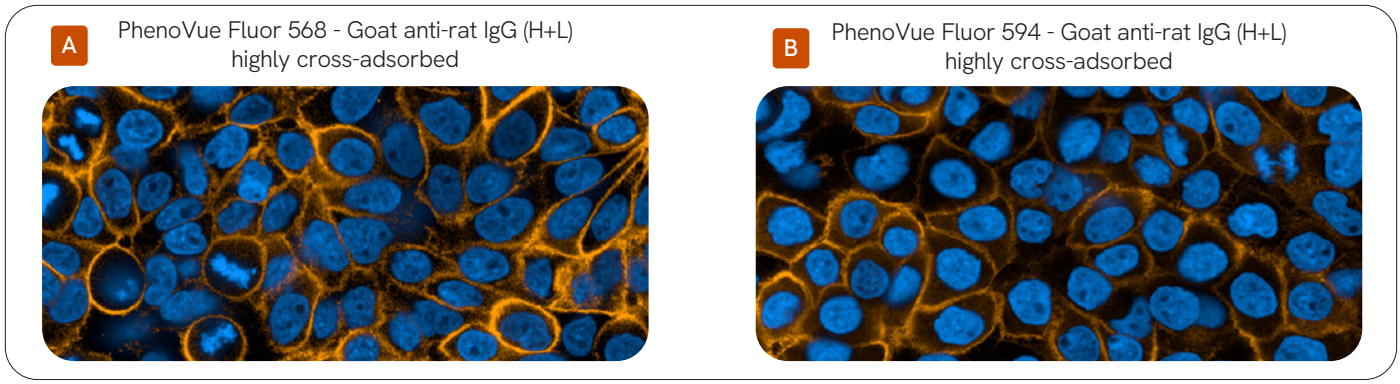



Figure 3: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% $CO_2$ for 24h. Cells were fixed then permeabilized and incubated with an anti-EGFR rat IgG2a antibody (2 µg/mL). After washing steps, cells were incubated with 10 µg/mL of PhenoVue Fluor 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.

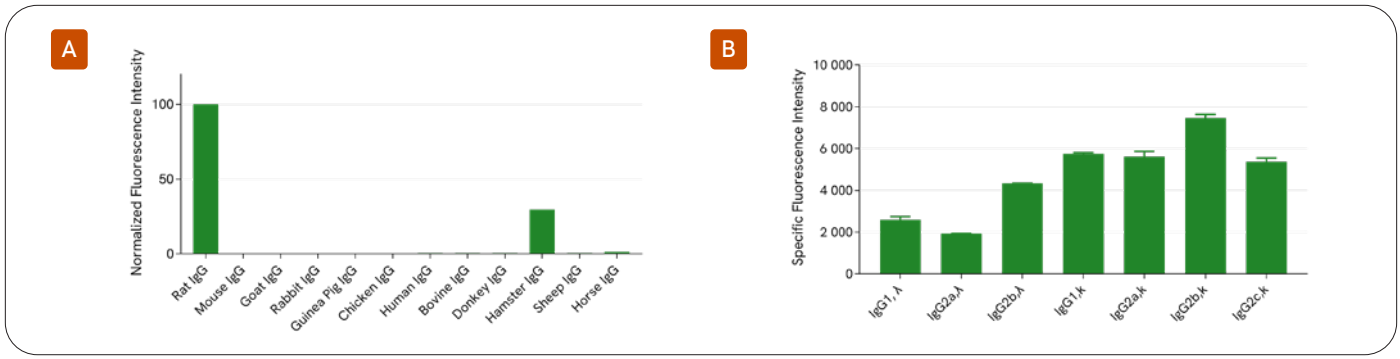



Figure 4: F-LISA experiments: different IgG species (A) or rat IgG isotypes (B) were used to coat a 96-well microplate, then incubated with PhenoVue Fluor 647 - Goat anti-rat IgG (H+L) highly cross-adsorbed (5 µg/mL). Fluorescence intensity was measured on an EnVision multimode plate reader.

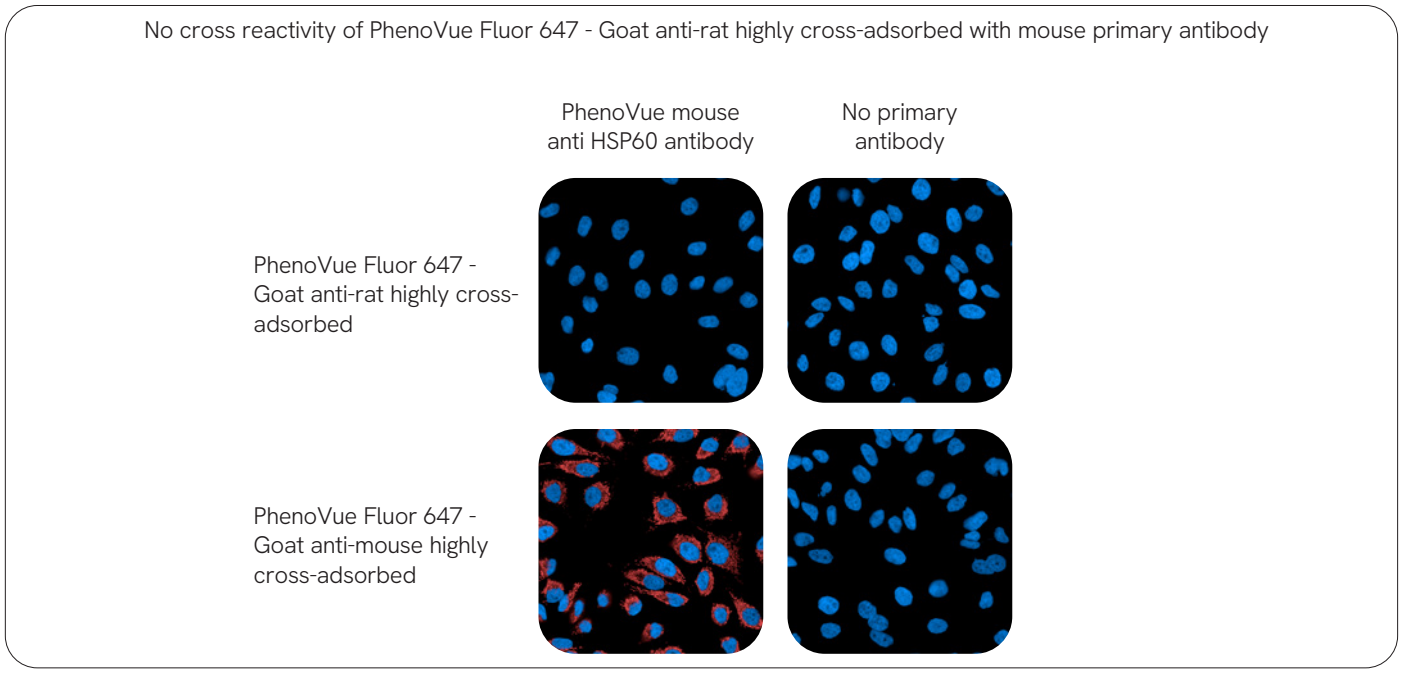



Figure 5: Hela cells were seeded in PhenoPlate 96-well microplates (35,000 cells/well) and incubated at 37 °C, 5% $CO_2$ for 24h. Cells were fixed then permeabilized and incubated with PhenoVue anti-HSP60 mouse antibody (1X) over night at 4 °C. After washing steps, cells were incubated with PhenoVue 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.

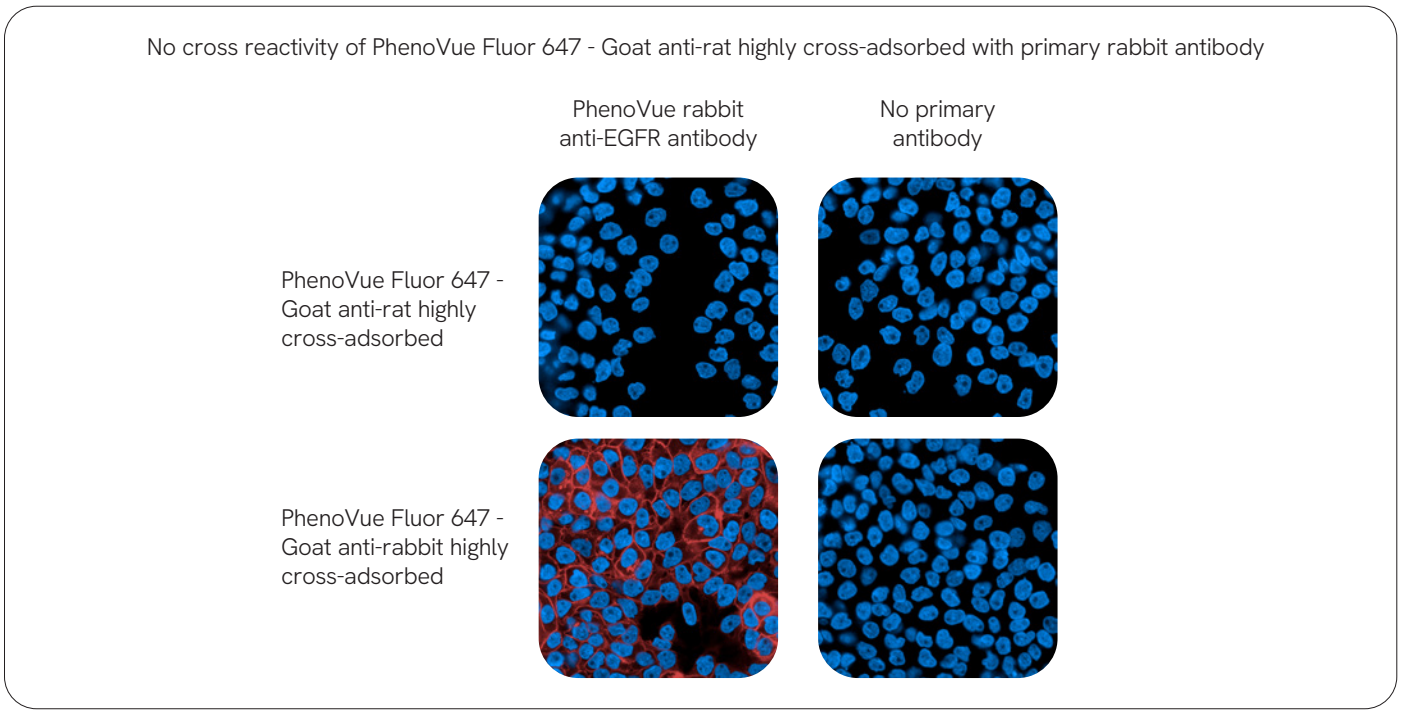



Figure 6: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% $CO_2$ for 24h. Cells were fixed then permeabilized and incubated with anti EGFR rabbit antibody (2µg/mL) (Panel B) 3h at RT. After washing steps, cells were incubated with PhenoVue Hoechst 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.

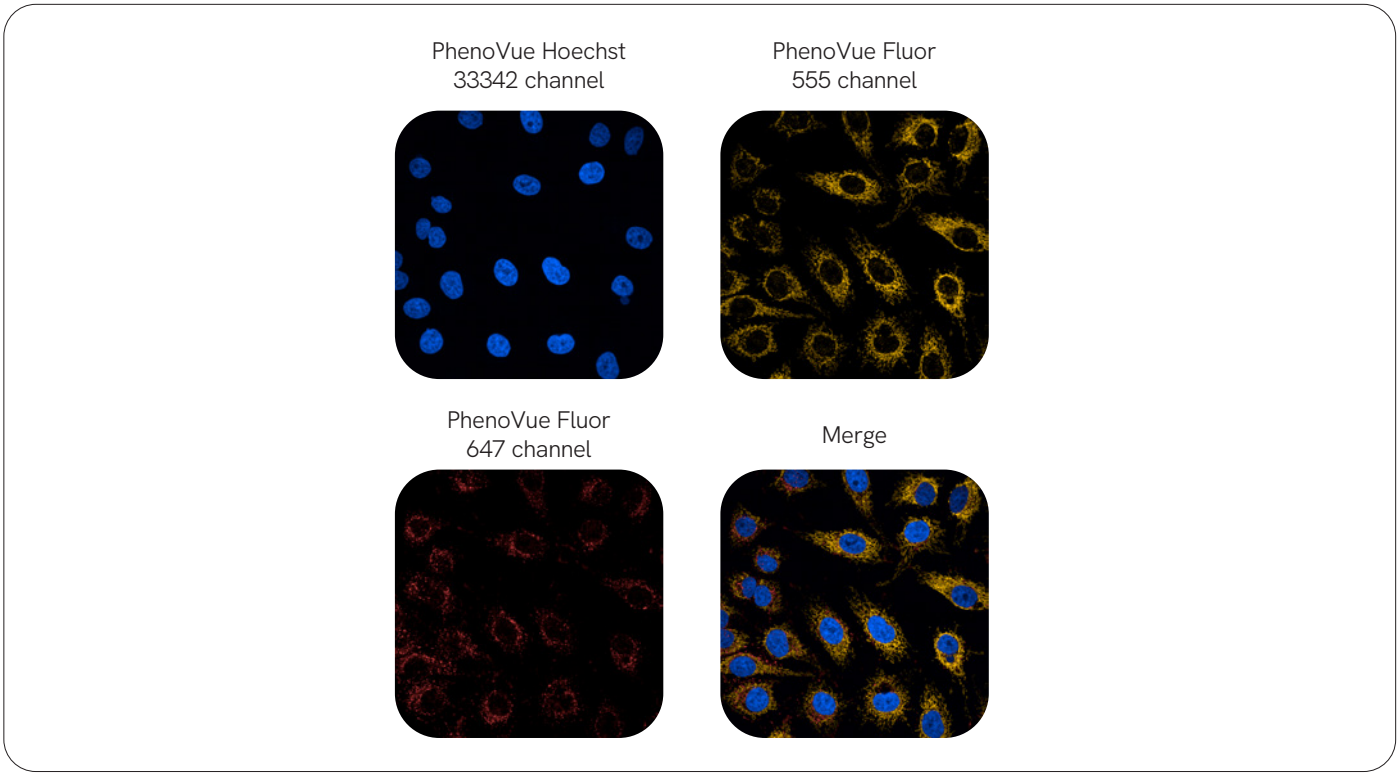



Figure 7: Hela cells were seeded in PhenoPlate 96-well microplates (35,000 cells/well) and incubated at 37 °C, 5% $CO_2$ for 24h. Cells were fixed then permeabilized and incubated with PhenoVue anti- HSP60 mouse antibody (1X) and PhenoVue anti-LAMP1 IgG2bκ rat antibody (1X) over night at 4 °C. After 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.

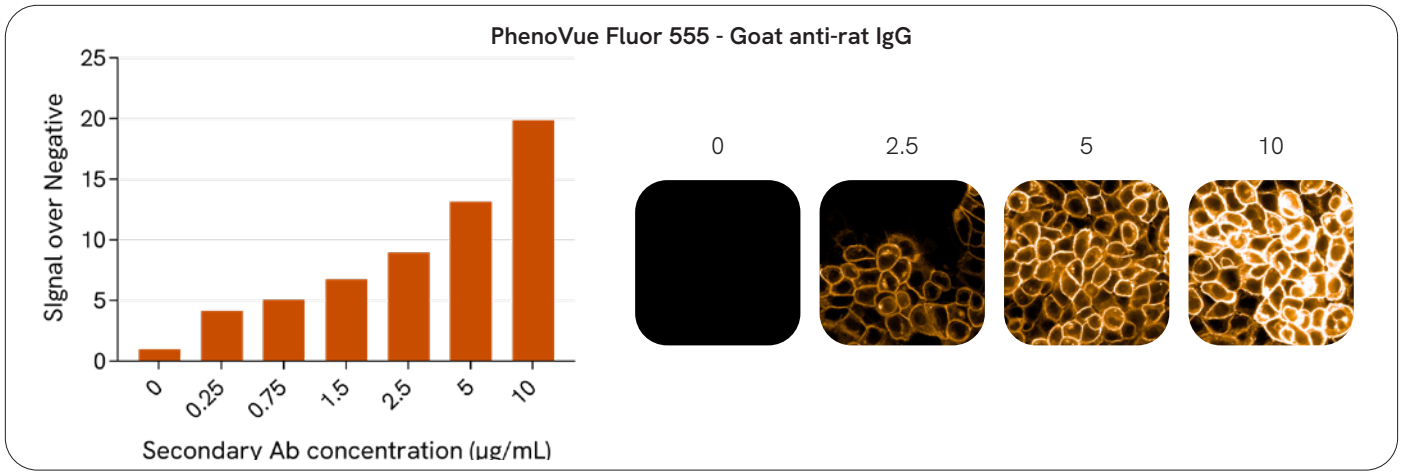



Figure 8: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% $CO_2$ for 24h. Cells were fixed then permeabilized and incubated with anti EGFR rat IgG2a antibody (2 µg/mL) (Panel B) 3h at RT. After washing steps, cells were incubated with PhenoVue Hoechst 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 1800 high-content analysis system using 63x water objective, confocal mode.

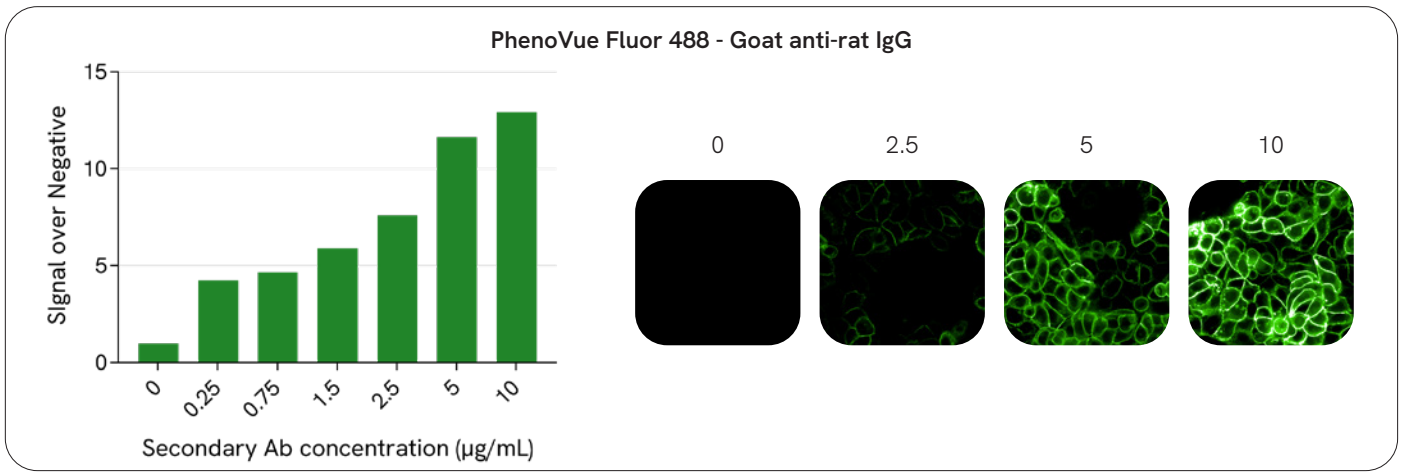



Figure 9: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% $CO_2$ for 24h. Cells were fixed then permeabilized and incubated with anti EGFR rat IgG2a antibody (2 µg/mL) (Panel B) 3h at RT. After washing steps, cells were incubated with PhenoVue Hoechst 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.

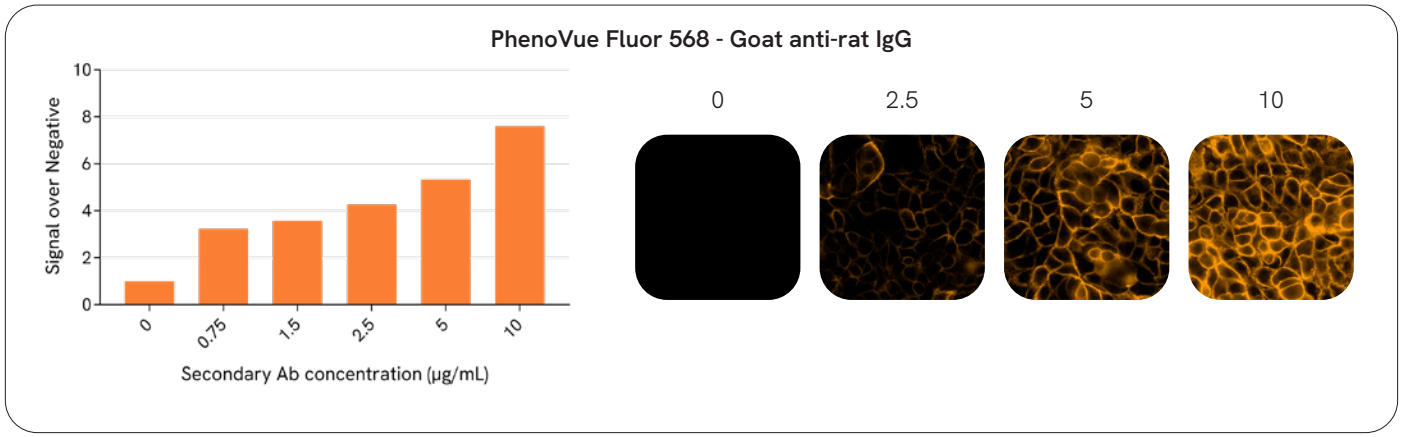



Figure 10: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% $CO_2$ for 24h. Cells were fixed then permeabilized and incubated with anti EGFR rat IgG2a antibody (2 µg/mL) (Panel B) 3h at RT. After washing steps, cells were incubated with PhenoVue 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.

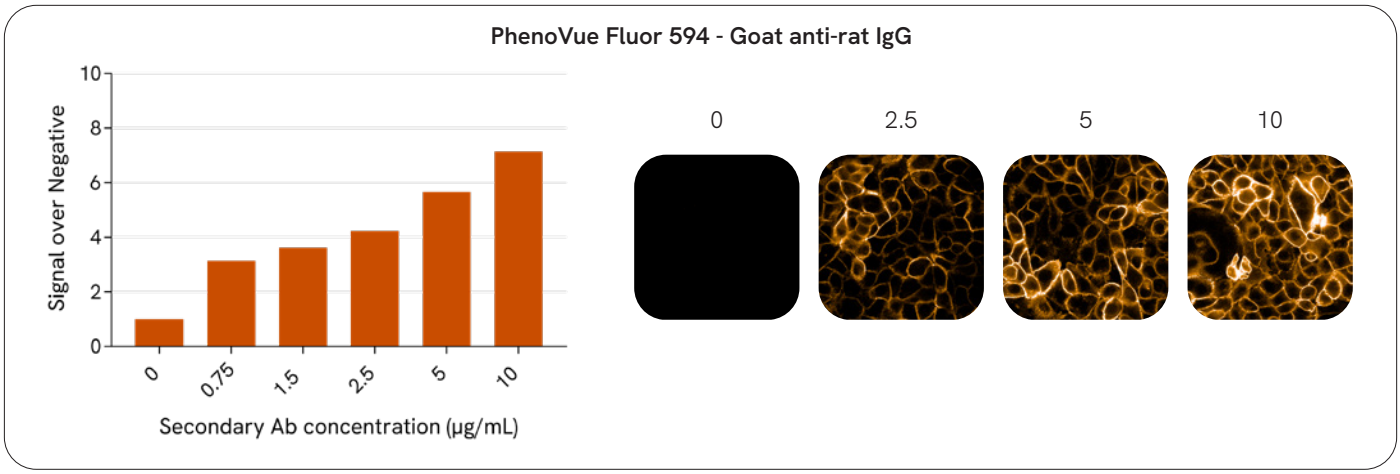



Figure 11: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% $CO_2$ for 24h. Cells were fixed then permeabilized and incubated with anti EGFR rat IgG2a antibody (2 µg/mL) (Panel B) 3h at RT. After washing steps, cells were incubated with PhenoVue 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.

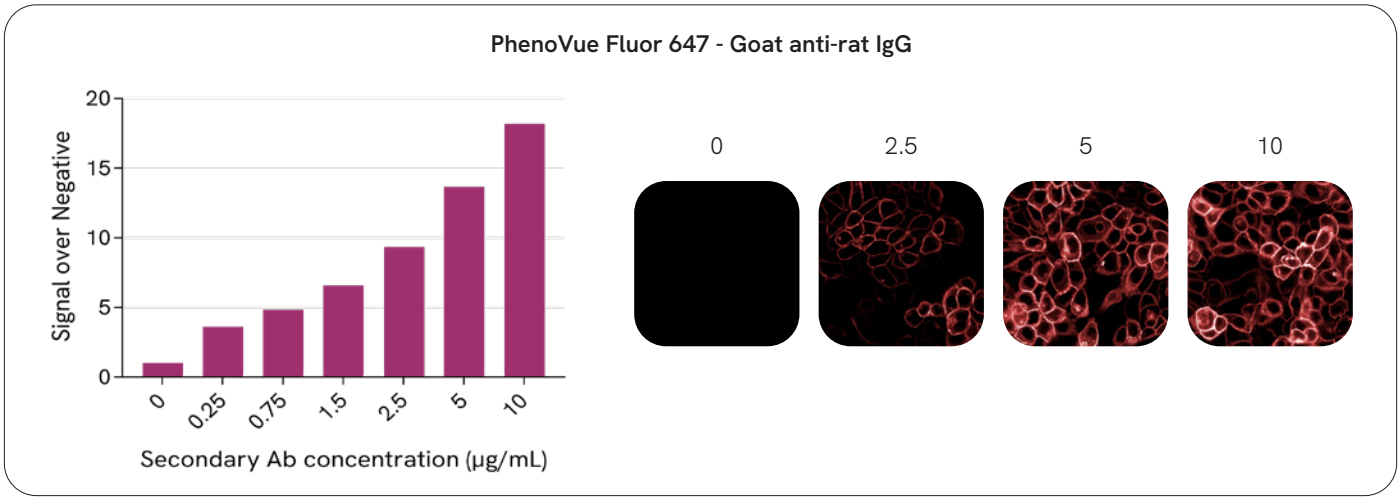



Figure 12: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% $CO_2$ for 24h. Cells were fixed then permeabilized and incubated with anti EGFR rat IgG2a antibody (2 µg/mL) (Panel B) 3h at RT. After washing steps, cells were incubated with PhenoVue 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.

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