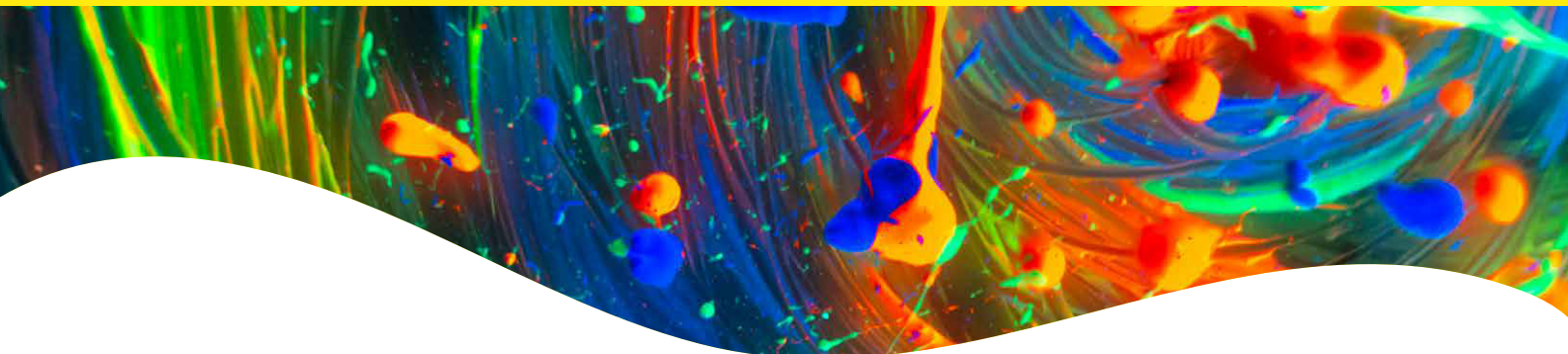


# PhenoVue Fluor - Goat anti-Mouse IgG (H+L) Antibody Conjugates

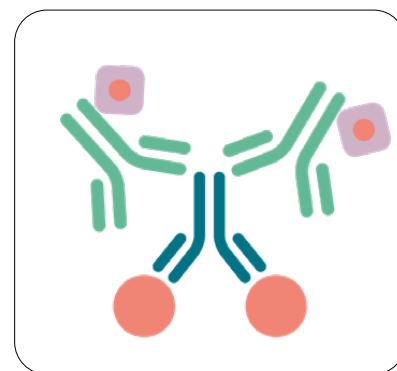


## Overview

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

PhenoVue Fluor dyes Goat anti-mouse IgG (H+L) antibodies, Cross-adsorbed are affinity purified and recognize different mouse IgG isotypes and may display cross-reactivity with other species such as rat or hamster.

PhenoVue Fluor dyes Goat anti-mouse IgG (H+L) antibodies, Highly Cross-adsorbed have been adsorbed against various IgG species such as rat, hamster, bovine, horse, human, rabbit, or chicken to minimize cross-reactivity.



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-Mouse antibody Cross-adsorbed          | 2GXM488C1   | 1                         | 1 mg              | Lyophilized | RT                  |
| PhenoVue Fluor 555 - Goat anti-Mouse antibody Cross-adsorbed          | 2GXM555C1   |                           |                   |             |                     |
| PhenoVue Fluor 568 - Goat anti-Mouse antibody Cross-adsorbed          | 2GXM568C1   |                           |                   |             |                     |
| PhenoVue Fluor 594 - Goat anti-Mouse antibody Cross-adsorbed          | 2GXM594C1   |                           |                   |             |                     |
| PhenoVue Fluor 647 - Goat anti-Mouse antibody Cross-adsorbed          | 2GXM647C1   |                           |                   |             |                     |
| PhenoVue Fluor 400LS - Goat anti-Mouse antibody Highly Cross-adsorbed | 2GXM400LSH1 | 1                         | 1 mg              | Lyophilized | RT                  |
| PhenoVue Fluor 405 - Goat anti-Mouse antibody Highly Cross-adsorbed   | 2GXM405H1   |                           |                   |             |                     |
| PhenoVue Fluor 488 - Goat anti-Mouse antibody Highly Cross-adsorbed   | 2GXM488H1   |                           |                   |             |                     |
| PhenoVue Fluor 555 - Goat anti-Mouse antibody Highly Cross-adsorbed   | 2GXM555H1   |                           |                   |             |                     |
| PhenoVue Fluor 568 - Goat anti-Mouse antibody Highly Cross-adsorbed   | 2GXM568H1   |                           |                   |             |                     |
| PhenoVue Fluor 594 - Goat anti-Mouse antibody Highly Cross-adsorbed   | 2GXM594H1   |                           |                   |             |                     |
| PhenoVue Fluor 647 - Goat anti-Mouse antibody Highly Cross-adsorbed   | 2GXM647H1   |                           |                   |             |                     |

## 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-Mouse antibody Cross-adsorbed          | 150000 g/mol     | Reconstitution using 1 mL ddH <sub>2</sub> O 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 555 - Goat anti-Mouse antibody Cross-adsorbed          |                  |   |   |
| PhenoVue Fluor 568 - Goat anti-Mouse antibody Cross-adsorbed          |                  |   |   |
| PhenoVue Fluor 594 - Goat anti-Mouse antibody Cross-adsorbed          |                  |   |   |
| PhenoVue Fluor 647 - Goat anti-Mouse antibody Cross-adsorbed          |                  |   |   |
| PhenoVue Fluor 400LS - Goat anti-Mouse antibody Highly Cross-adsorbed | 150000 g/mol     | Reconstitution using 1 mL ddH <sub>2</sub> O 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-Mouse antibody Highly Cross-adsorbed   |                  |   | 1 µg/mL - 10 µg/mL (6.7 nM - 66.7 nM)   |
| PhenoVue Fluor 488 - Goat anti-Mouse antibody Highly Cross-adsorbed   |                  |   |   |
| PhenoVue Fluor 555 - Goat anti-Mouse antibody Highly Cross-adsorbed   |                  |   |   |
| PhenoVue Fluor 568 - Goat anti-Mouse antibody Highly Cross-adsorbed   |                  |   |   |
| PhenoVue Fluor 594 - Goat anti-Mouse antibody Highly Cross-adsorbed   |                  |   |   |
| PhenoVue Fluor 647 - Goat anti-Mouse 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-Mouse antibody Cross-adsorbed          | 2.5 µg/mL (16.7 nM)                    | Approx. 14-42                                 | Approx. 14-42                                | Approx. 22-65                                |
| PhenoVue Fluor 555 - Goat anti-Mouse antibody Cross-adsorbed          |  |   |  |  |
| PhenoVue Fluor 568 - Goat anti-Mouse antibody Cross-adsorbed          |  |   |  |  |
| PhenoVue Fluor 594 - Goat anti-Mouse antibody Cross-adsorbed          |  |   |  |  |
| PhenoVue Fluor 647 - Goat anti-Mouse antibody Cross-adsorbed          |  |   |  |  |
| PhenoVue Fluor 400LS - Goat anti-Mouse 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-Mouse 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-Mouse antibody Highly Cross-adsorbed   | 2.5 µg/mL (16.7 nM)                    | Approx. 14-42                                 | Approx. 14-42                                | Approx. 22-65                                |
| PhenoVue Fluor 555 - Goat anti-Mouse antibody Highly Cross-adsorbed   |  |   |  |  |
| PhenoVue Fluor 568 - Goat anti-Mouse antibody Highly Cross-adsorbed   |  |   |  |  |
| PhenoVue Fluor 594 - Goat anti-Mouse antibody Highly Cross-adsorbed   |  |   |  |  |
| PhenoVue Fluor 647 - Goat anti-Mouse antibody Highly Cross-adsorbed   |  |   |  |  |

View our full range of high-quality imaging microplates at [Revvity.com](http://Revvity.com)

## Spectral and photophysical properties

| Product name         | Maximum excitation wavelength (nm) | Maximum emission wavelength (nm) | Common filter set                | Quantum yield ( $\Phi$ ) | Epsilon* ( $\epsilon$ in $M^{-1}\cdot cm^{-1}$ at $\lambda$ max) | Brightness ( $\Phi \times \epsilon$ ) |
|----------------------|------------------------------------|----------------------------------|----------------------------------|--------------------------|--|---------------------------------------|
| PhenoVue Fluor 400LS | 395                                | 585                              | Ex: 375-440<br>Em: 550-650       | nd*                      | 26000  | nd*                                   |
| PhenoVue Fluor 405   | 410                                | 452                              | Ex: 375-430 nm<br>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                                | 617                              | 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             | 425                | 425                  | 488                | 561                | 561                                      | 640                |
|                            | Emission filter              | 435-480            | 570-630              | 500-550            | 570-630            | 570-630                                  | 650-760            |
| Opera Phenix Plus 4 lasers | Excitation laser             | 405                | 405                  | 488                | 561                | 561                                      | 640                |
|                            | Emission filter              | 435-480            | 570-630              | 500-550            | 570-630            | 570-630                                  | 650-760            |
| Operetta CLS 8 LED - 1600  | Excitation LED (filter) (nm) | 405 (390-420)      | 405 (390-420)        | 475 (460-490)      | 550 (530-560)      | 550 (530-560)                            | 630 (615-645)      |
|                            | Emission filter              | 430-500            | 570-650              | 500-550            | 570-650            | 570-650                                  | 655-760            |
| Operetta CLS 8 LED - 1601  | Excitation LED (filter) (nm) | 370 (355-385)      | 440 (435-460)        | 475 (460-490)      | 550 (530-560)      | 580 (560-575)                            | 630 (615-645)      |
|                            | Emission filter              | 430-500            | 600-640 or 570-650   | 500-550            | 570-650            | 585-640                                  | 655-760            |
| Operetta CLS 4 LED         | Excitation LED (filter) (nm) | 370 (355-385)      | 370 (355-385)        | 475 (460-490)      | 550 (530-560)      | 550 (530-560)                            | 630 (615-645)      |
|                            | Emission filter              | 430-500            | 570-650              | 500-550            | 570-650            | 570-650                                  | 655-760            |

## Cross-reactivity

| Product name  | Across species  | Across IgG isotypes   |
|---|---|---|
| PhenoVue Fluor 488 - Goat anti-Mouse antibody Cross-adsorbed          | Mouse:100%<br>Rat: 44%<br>Guinea Pig: 16%<br>Hamster: 50%<br>Horse: 10% | Cross-reactivity with mouse:<br>IgG1<br>IgG2a<br>IgG2b<br>IgG2c<br>IgG3 |
| PhenoVue Fluor 555 - Goat anti-Mouse antibody Cross-adsorbed          |   |   |
| PhenoVue Fluor 568 - Goat anti-Mouse antibody Cross-adsorbed          |   |   |
| PhenoVue Fluor 594 - Goat anti-Mouse antibody Cross-adsorbed          |   |   |
| PhenoVue Fluor 647 - Goat anti-Mouse antibody Cross-adsorbed          |   |   |
| PhenoVue Fluor 400LS - Goat anti-Mouse antibody Highly Cross-adsorbed | Mouse:100%  | Cross-reactivity with mouse:<br>IgG1<br>IgG2a<br>IgG2b<br>IgG2c<br>IgG3 |
| PhenoVue Fluor 405 - Goat anti-Mouse antibody Highly Cross-adsorbed   |   |   |
| PhenoVue Fluor 488 - Goat anti-Mouse antibody Highly Cross-adsorbed   |   |   |
| PhenoVue Fluor 555 - Goat anti-Mouse antibody Highly Cross-adsorbed   |   |   |
| PhenoVue Fluor 568 - Goat anti-Mouse antibody Highly Cross-adsorbed   |   |   |
| PhenoVue Fluor 594 - Goat anti-Mouse antibody Highly Cross-adsorbed   |   |   |
| PhenoVue Fluor 647 - Goat anti-Mouse 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<sub>2</sub> 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 mouse antibody.

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

#### 8. Staining: Incubate with 1-10 µg/mL PhenoVue Fluor Goat anti-Mouse antibody Cross-adsorbed or 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 10min 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 365nm excitation (e.g Operetta CLS - 4LED), 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

- Due to species cross-reactivity of PhenoVue Goat anti-Mouse cross-adsorbed antibodies, especially with rodents, it is preferable to use PhenoVue Goat anti-Mouse highly cross-adsorbed antibodies when performing multiplexing experiments including different primary antibodies (see Figures 9, 10 and 11). Please note that this is not limited to PhenoVue secondary antibodies but rather a general characteristic of antibodies irrespective of the vendor.
- PhenoVue Goat anti-Mouse cross-adsorbed antibodies are well suited for single-plex experiments.

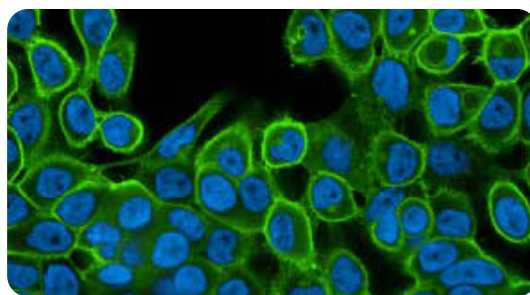
## Safety information

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-Mouse IgG (H+L)  
Cross-adsorbed

Figure 1: A431 cells were seeded in PhenoPlate™ 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO<sub>2</sub> for 24h. Cells were fixed then permeabilized and incubated with an anti-EGFR Mouse antibody (0.2 µg/mL). After washing steps, cells were incubated with 10 µg/mL of PhenoVue Fluor 488 - Goat anti-Mouse IgG (H+L) 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.

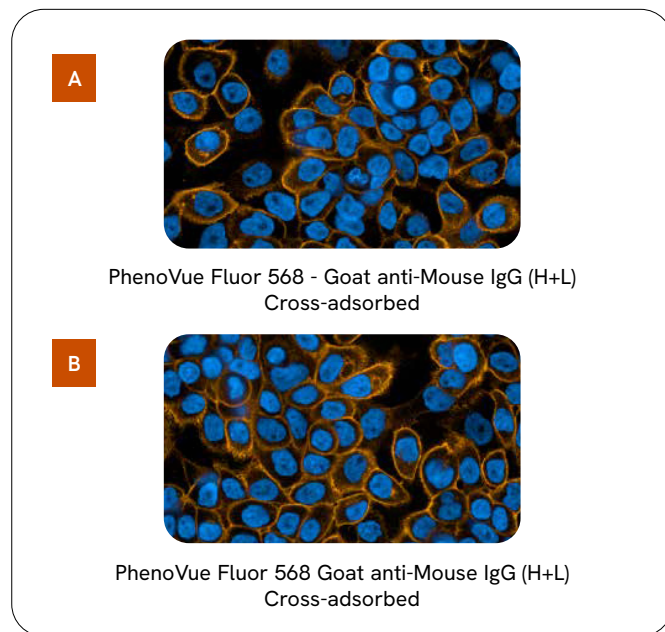
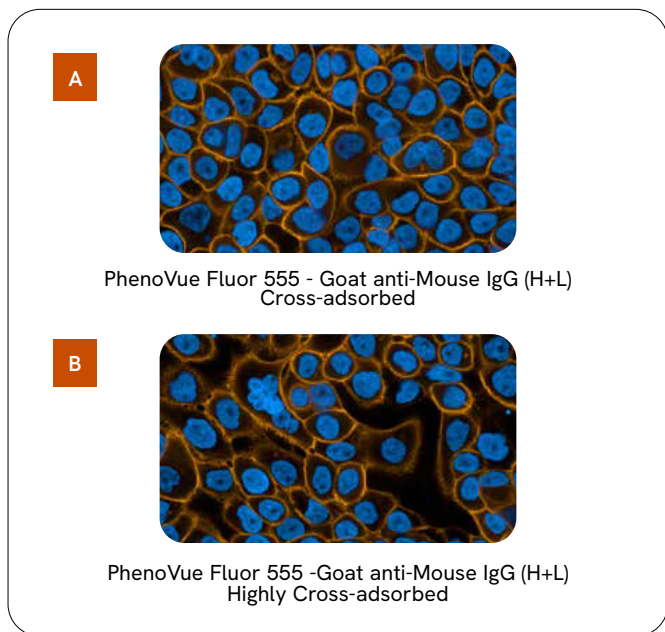


Figure 2: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO<sub>2</sub> for 24h. Cells were fixed then permeabilized and incubated with an anti-EGFR Mouse antibody (0.2 µg/mL). After washing steps, cells were incubated with 10 µg/mL of PhenoVue Fluor 555 - Goat anti-Mouse IgG (H+L) Cross-adsorbed (A) or 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.

Figure 3: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO<sub>2</sub> for 24h. Cells were fixed then permeabilized and incubated with an anti-EGFR Mouse antibody (0.2 µg/mL). After washing steps, cells were incubated with 10 µg/mL of PhenoVue Fluor 568 - Goat anti-Mouse IgG (H+L) Cross-adsorbed (A) or 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.

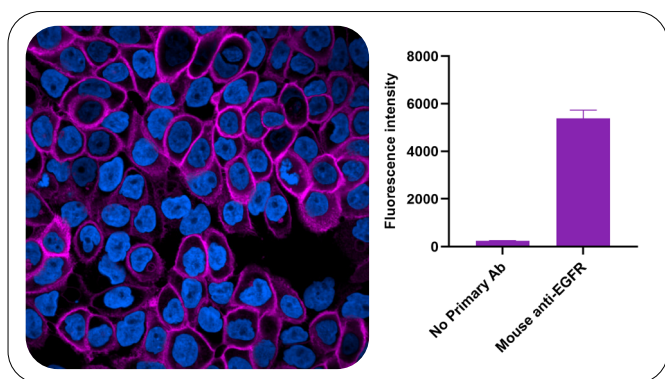


Figure 4: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO<sub>2</sub> for 24h. Cells were fixed then permeabilized and incubated with an anti-EGFR mouse antibody (2 µg/mL). After washing steps, cells were incubated with 20 µg/mL of PhenoVue Fluor 400LS - Goat anti-mouse 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.

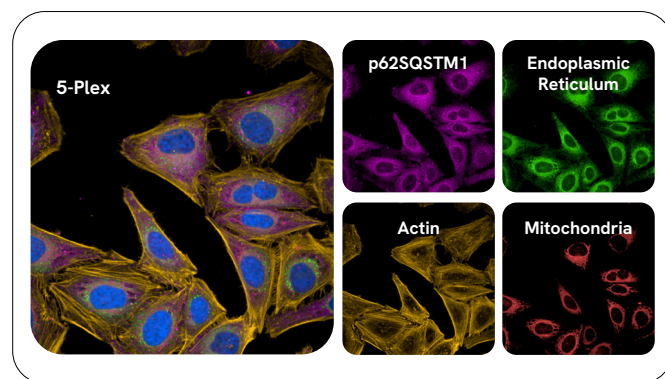


Figure 5: U2OS cells were seeded in PhenoPlate 96-well microplates (15,000 cells/well) and incubated at 37 °C, 5% CO<sub>2</sub> for 24h. Live cells were first stained for 30 minutes with 500nM of PhenoVue 641 mitochondrial stain before fixation, permeabilization and saturation steps. Cells were then incubated overnight with mouse anti-p62SQSTM1 (5µg/mL). After washing steps, a staining mix with 20 µg/mL of PhenoVue Fluor 400LS - goat anti-mouse IgG (H+L) highly cross-adsorbed, 50ng/mL of PhenoVue Hoechst, 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.

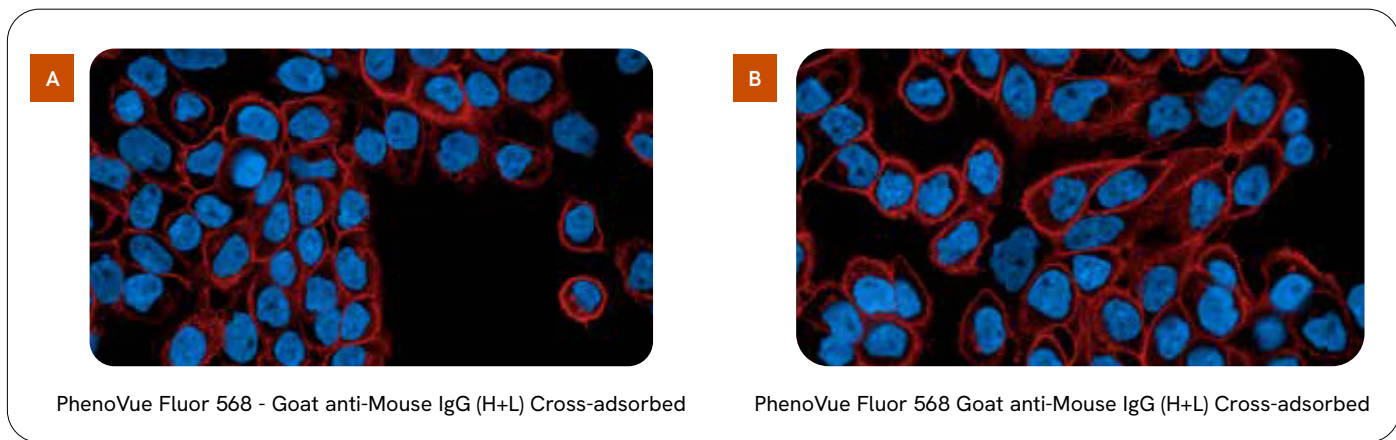


Figure 6: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37°C, 5% CO<sub>2</sub> for 24h. Cells were fixed then permeabilized and incubated with an anti-EGFR Mouse antibody (0.2 µg/mL). After washing steps, cells were incubated with 10 µg/mL of PhenoVue Fluor 594 - Goat anti-Mouse IgG (H+L) Cross-adsorbed (A) or 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.

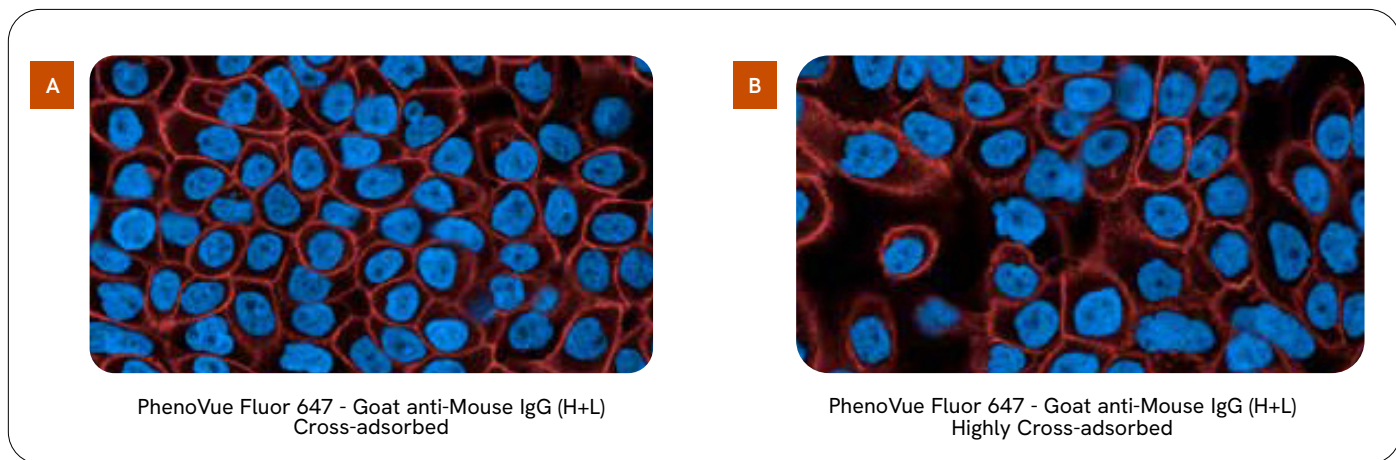


Figure 7: A431 cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37°C, 5% CO<sub>2</sub> for 24h. Cells were fixed then permeabilized and incubated with an anti-EGFR Mouse antibody (0.2 µg/mL). After washing steps, cells were incubated with 10 µg/mL of PhenoVue Fluor 647 - Goat anti-Mouse IgG (H+L) Cross-adsorbed (A) or 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.

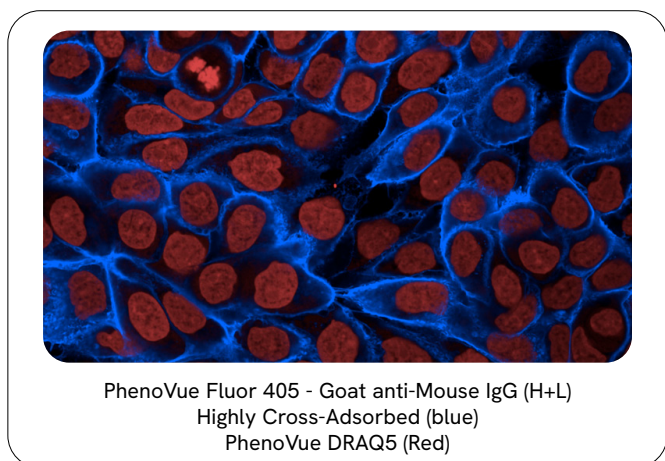


Figure 8: A431 cells were seeded in PhenoPlate™ 96-well microplates at a density of 75,000 cells per well and incubated for 24 hours at 37°C with 5% CO<sub>2</sub>. After incubation, the cells were fixed, permeabilized, and treated with a 2 µg/mL concentration of anti-EGFR mouse antibody. Following washing steps, the cells were incubated with 10 µg/mL PhenoVue Fluor 405 - Goat anti-Mouse 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 captured using the Opera Phenix Plus high-content analysis system with 5 lasers, using a 63x water objective in confocal mode.

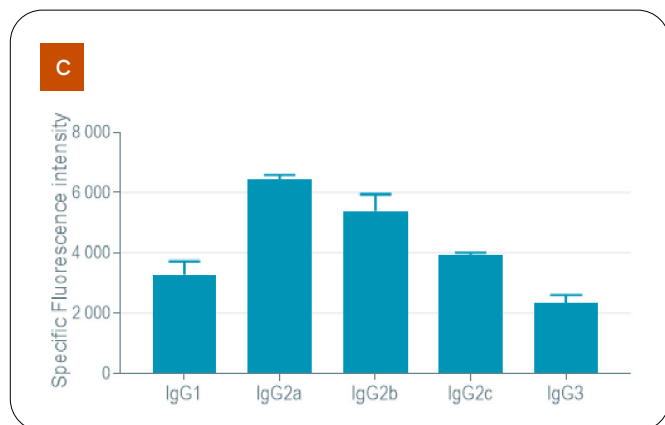
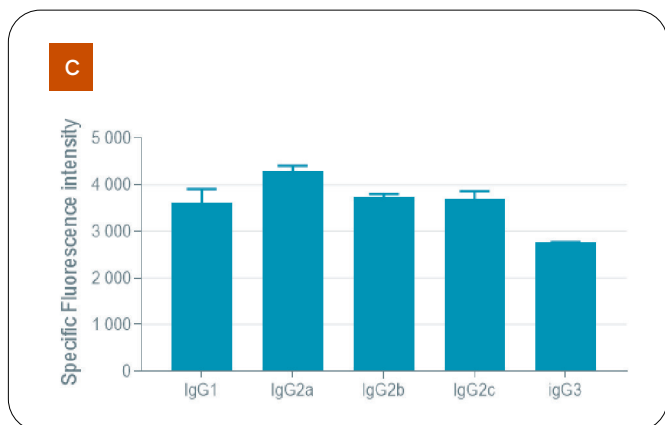
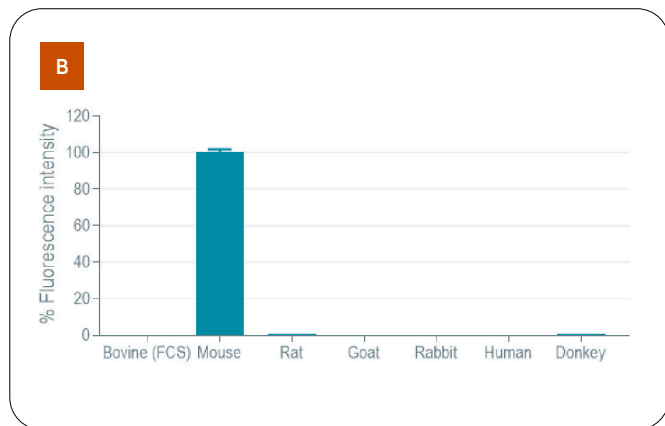
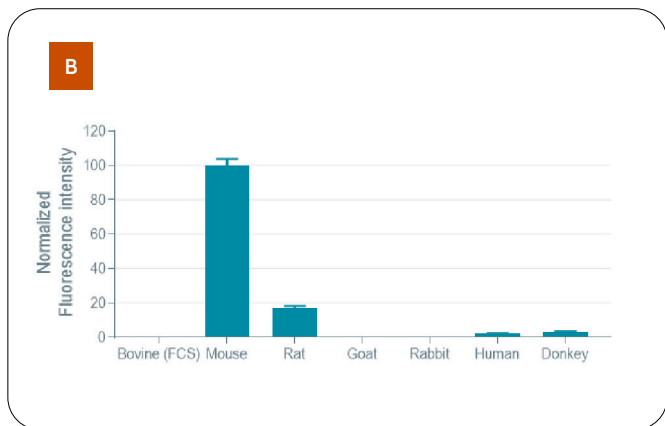
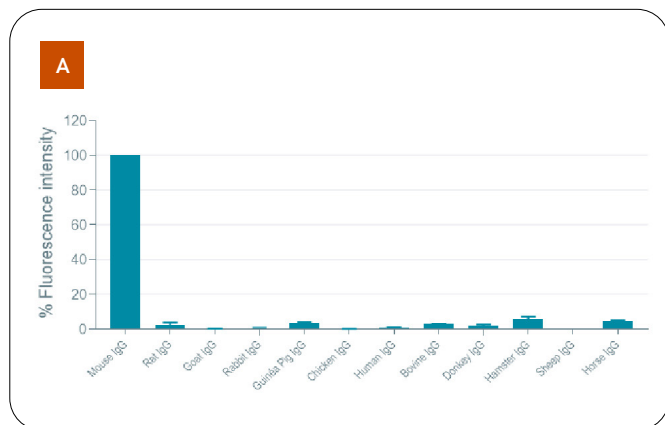
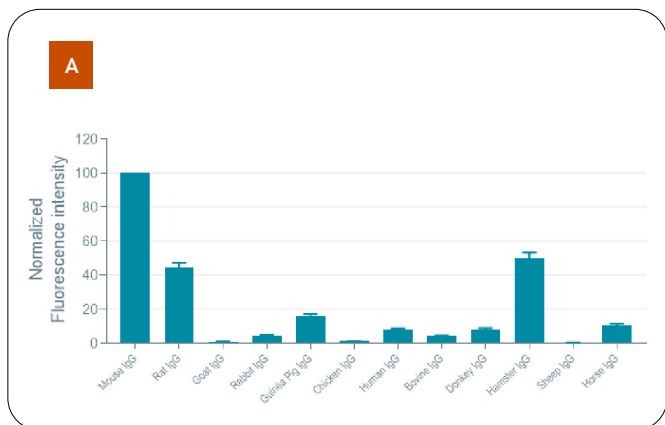


Figure 9.1: F-LISA experiments: different IgG species (A), 10% of serum (B) or IgG isotypes (C) were used to coat a 96-well microplate, then incubated with PhenoVue Fluor 594 - Goat anti-Mouse IgG (H+L) Cross-Adsorbed (1.5 µg/mL). Fluorescence intensity was measured on an EnVision™ multimode plate reader.

Figure 9.2: F-LISA experiments: different IgG species (A), 10% of serum (B) or IgG isotypes (C) were used to coat a 96-well microplate, then incubated with PhenoVue Fluor 594 - Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed (1.5 µg/mL). Fluorescence Intensity was measured on an EnVision multimode plate reader.



No Cross reactivity of PhenoVue Fluor 488 - Goat anti-Mouse Cross-Adsorbed with primary Rabbit antibody

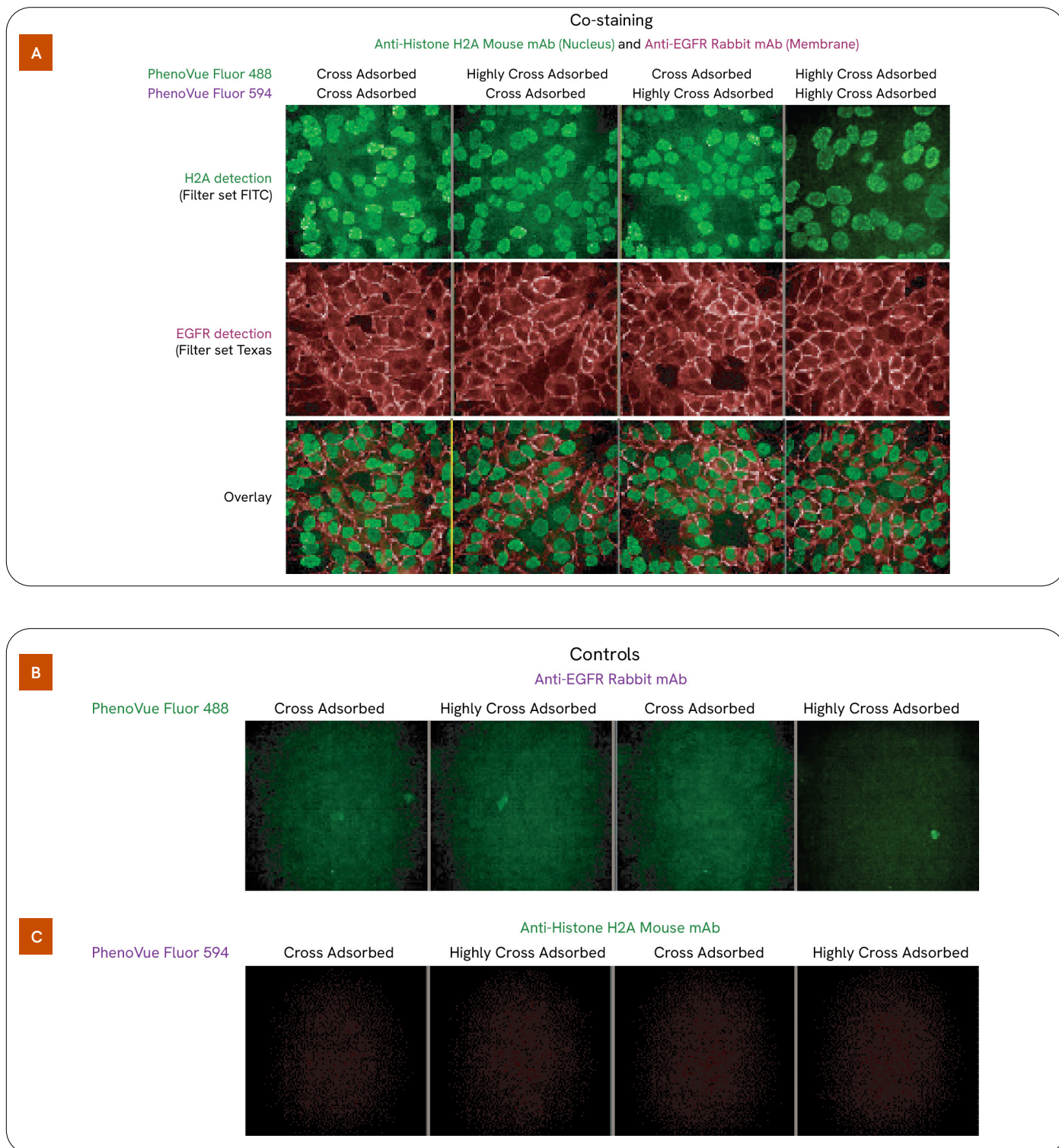


Figure 10: Hela cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well) and incubated at 37 °C, 5% CO<sub>2</sub> for 24h. Cells were fixed then permeabilized and co-incubated with anti- Histone H2A mouse antibody (5 µg/mL) and anti-EGFR rabbit antibody (0.5 µg/mL). After washing steps, cells were incubated with 1.5 µg/mL PhenoVue Fluor 488 - Goat anti-Mouse IgG (H+L) Cross-Adsorbed or Highly Cross-adsorbed and PhenoVue Fluor 594 - Goat anti-Rabbit IgG (H+L) Cross-Adsorbed or Highly Cross-adsorbed for 1 hour at RT (Panel A). Cross reactivity of PhenoVue Fluor 488 - Goat anti-Mouse antibody either Cross-Adsorbed or Highly Cross-adsorbed was assessed by incubating anti-EGFR rabbit antibody only (Panel B). Cross reactivity of PhenoVue Fluor 594 - Goat anti-Rabbit antibody either Cross-Adsorbed or Highly Crossadsorbed was assessed by incubating anti-Histone H2A mouse antibody only (Panel C). Images were acquired on the Operetta CLS high-content analysis system.

No Cross reactivity of PhenoVue Fluor 488 - Goat anti-Mouse Highly Cross-Adsorbed with primary Rat antibody

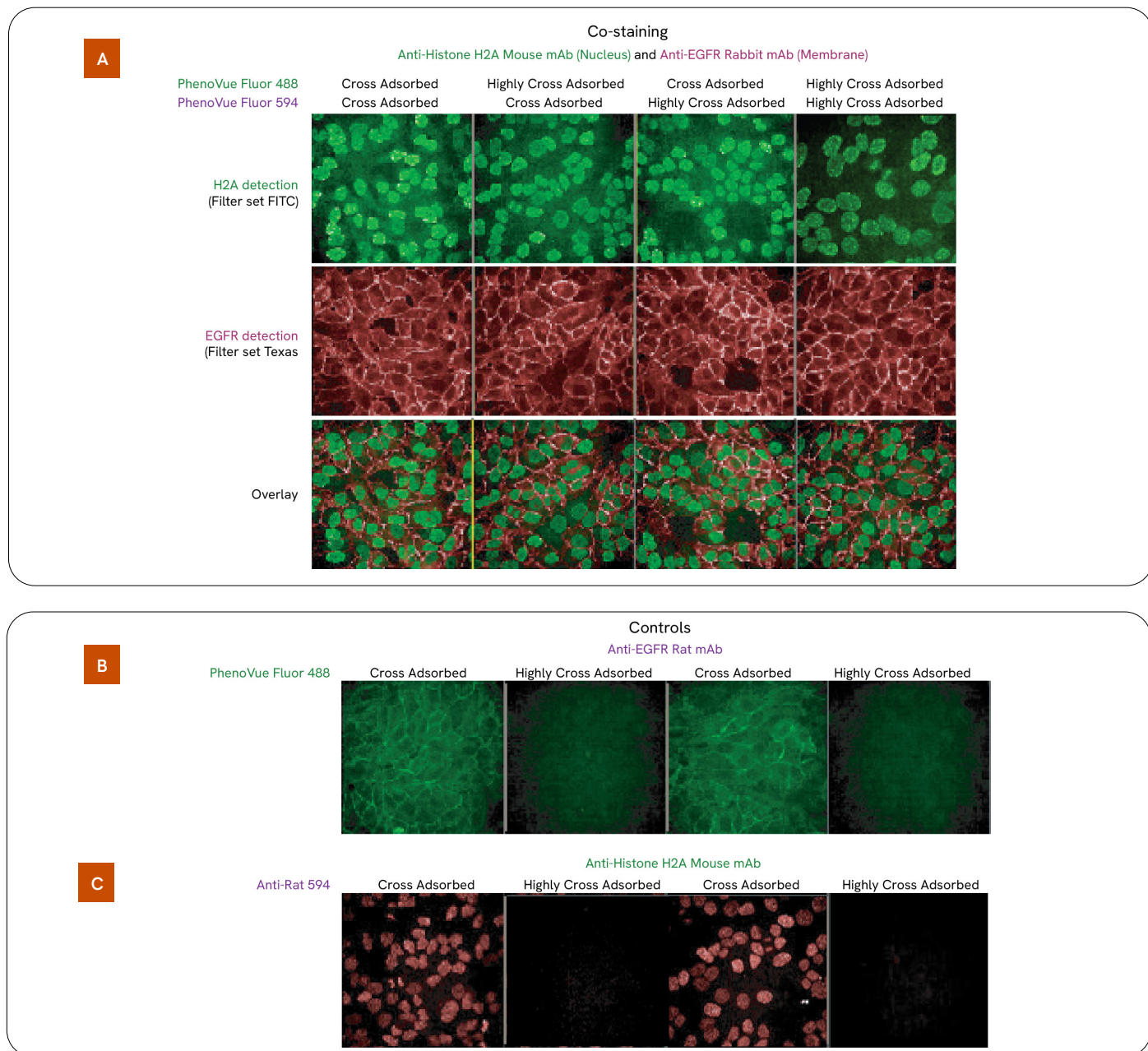


Figure 11: HeLa cells were seeded in PhenoPlate 96-well microplates (50,000 cells/well) and incubated at 37 °C, 5% CO<sub>2</sub> for 24h. Cells were fixed then permeabilized and co incubated with anti- Histone H2A mouse antibody (5 µg/mL) and anti-EGFR Rat antibody (1 µg/mL). After washing steps, cells were incubated with 1.5 µg/mL PhenoVue Fluor 488 - Goat anti-Mouse IgG (H+L) Cross-adsorbed or Highly Cross-adsorbed and Anti-Rat 594 Cross-adsorbed or Highly Cross-adsorbed for 1 hour at RT (Panel A). Cross reactivity of PhenoVue Fluor 488 - Goat anti-Mouse antibody either Cross-adsorbed or Highly Cross-adsorbed was assessed by incubating anti-EGFR rabbit antibody only (Panel B). Cross reactivity of Anti-Rat 594 either Cross-Adsorbed or Highly Cross-adsorbed was assessed by incubating anti-Histone H2A mouse antibody only (Panel C). Anti-Rat 594 is a fluor-labelled goat anti-rat second antibody with a maximum excitation wavelength of 594 nm, and maximum emission wavelength of 617 nm. Images were acquired on the Operetta CLS high-content analysis system.

