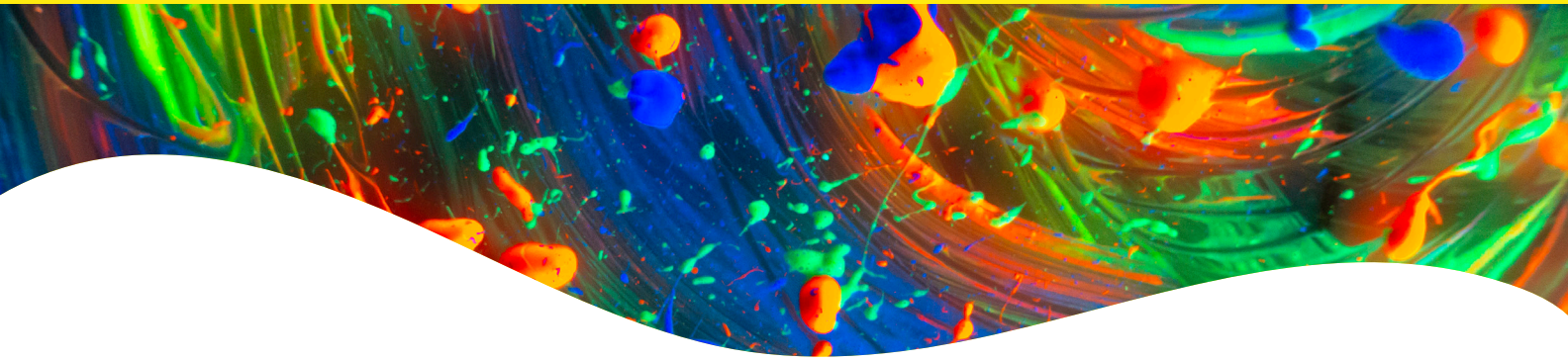
PhenoVue Fluor - Donkey anti-Mouse IgG (H+L) Antibody Conjugates

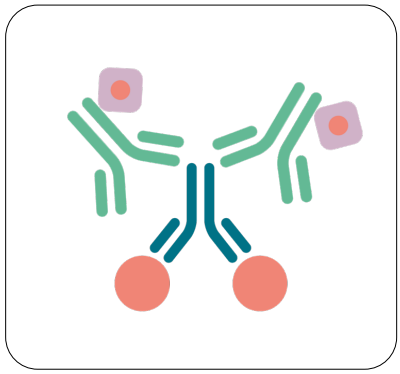


Overview

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

PhenoVue Fluor dyes Donkey 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 Donkey 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.	Number of vials per unit	Quantity per vial	Format	Shipping conditions
PhenoVue Fluor 488 - Donkey anti-Mouse antibody Cross-adsorbed	2DXM488C1	1	1 mg	Lyophilized	RT
PhenoVue Fluor 555 - Donkey anti-Mouse antibody Cross-adsorbed	2DXM555C1				
PhenoVue Fluor 568 - Donkey anti-Mouse antibody Cross-adsorbed	2DXM568C1				
PhenoVue Fluor 594 - Donkey anti-Mouse antibody Cross-adsorbed	2DXM594C1				
PhenoVue Fluor 647 - Donkey anti-Mouse antibody Cross-adsorbed	2DXM647C1				
PhenoVue Fluor 488 - Donkey anti-Mouse antibody Highly Cross-adsorbed	2DXM488H1	1	1 mg	Lyophilized	RT
PhenoVue Fluor 555 - Donkey anti-Mouse antibody Highly Cross-adsorbed	2DXM555H1				
PhenoVue Fluor 568 - Donkey anti-Mouse antibody Highly Cross-adsorbed	2DXM568H1				
PhenoVue Fluor 594 - Donkey anti-Mouse antibody Highly Cross-adsorbed	2DXM594H1				
PhenoVue Fluor 647 - Donkey anti-Mouse antibody Highly Cross-adsorbed	2DXM647H1				

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 - Donkey anti-Mouse antibody Cross-adsorbed	150000 g/mol	Reconstitution using 1 mL ddH_2O gives a stock concentration of 1 mg/mL (6.66 µM)	0.1 µg/mL – 10 µg/mL (0.66 nM – 66.6 nM)
PhenoVue Fluor 555 - Donkey anti-Mouse antibody Cross-adsorbed			
PhenoVue Fluor 568 - Donkey anti-Mouse antibody Cross-adsorbed			
PhenoVue Fluor 594 - Donkey anti-Mouse antibody Cross-adsorbed			
PhenoVue Fluor 647 - Donkey anti-Mouse antibody Cross-adsorbed			
PhenoVue Fluor 488 - Donkey anti-Mouse antibody Highly Cross-adsorbed	150000 g/mol	Reconstitution using 1 mL ddH_2O gives a stock concentration of 1 mg/mL (6.66 µM)	0.1 µg/mL – 10 µg/mL (0.66 nM – 66.6 nM)
PhenoVue Fluor 555 - Donkey anti-Mouse antibody Highly Cross-adsorbed			
PhenoVue Fluor 568 - Donkey anti-Mouse antibody Highly Cross-adsorbed			
PhenoVue Fluor 594 - Donkey anti-Mouse antibody Highly Cross-adsorbed			
PhenoVue Fluor 647 - Donkey 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 - Donkey anti-Mouse antibody Cross-adsorbed	1.5 µg/mL (10 nM)	Approx. 25-70	Approx. 20-70	Approx. 35-90
PhenoVue Fluor 555 - Donkey anti-Mouse antibody Cross-adsorbed				
PhenoVue Fluor 568 - Donkey anti-Mouse antibody Cross-adsorbed				
PhenoVue Fluor 594 - Donkey anti-Mouse antibody Cross-adsorbed				
PhenoVue Fluor 647 - Donkey anti-Mouse antibody Cross-adsorbed				
PhenoVue Fluor 488 - Donkey anti-Mouse antibody Highly Cross-adsorbed	1.5 µg/mL (10 nM)	Approx. 25-70	Approx. 20-70	Approx. 35-90
PhenoVue Fluor 555 - Donkey anti-Mouse antibody Highly Cross-adsorbed				
PhenoVue Fluor 568 - Donkey anti-Mouse antibody Highly Cross-adsorbed				
PhenoVue Fluor 594 - Donkey anti-Mouse antibody Highly Cross-adsorbed				
PhenoVue Fluor 647 - Donkey anti-Mouse antibody Highly Cross-adsorbed				

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	617	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 - Donkey anti-Mouse antibody Cross-adsorbed	Rat: 38% Guinea Pig: 13% Hamster: 44%	Cross-reactivity with mouse: IgG1 IgG2a IgG2b IgG2c IgG3
PhenoVue Fluor 555 - Donkey anti-Mouse antibody Cross-adsorbed		
PhenoVue Fluor 568 - Donkey anti-Mouse antibody Cross-adsorbed		
PhenoVue Fluor 594 - Donkey anti-Mouse antibody Cross-adsorbed		
PhenoVue Fluor 647 - Donkey anti-Mouse antibody Cross-adsorbed		
PhenoVue Fluor 488 - Donkey anti-Mouse antibody Highly Cross-adsorbed	None	Cross-reactivity with mouse: IgG1 IgG2a IgG2b IgG2c IgG3
PhenoVue Fluor 555 - Donkey anti-Mouse antibody Highly Cross-adsorbed		
PhenoVue Fluor 568 - Donkey anti-Mouse antibody Highly Cross-adsorbed		
PhenoVue Fluor 594 - Donkey anti-Mouse antibody Highly Cross-adsorbed		
PhenoVue Fluor 647 - Donkey 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_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 mouse antibody.

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

8. **Staining:** Incubate with 0.1-10 µg/mL PhenoVue Fluor Donkey anti-Mouse antibody Cross-adsorbed or 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

- Due to species cross-reactivity of PhenoVue Donkey anti-Mouse cross-adsorbed antibodies, especially with rodents, it is preferable to use PhenoVue Donkey anti-Mouse 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.
- PhenoVue Donkey anti-Mouse cross-adsorbed antibodies are well suited for single-plex experiments.

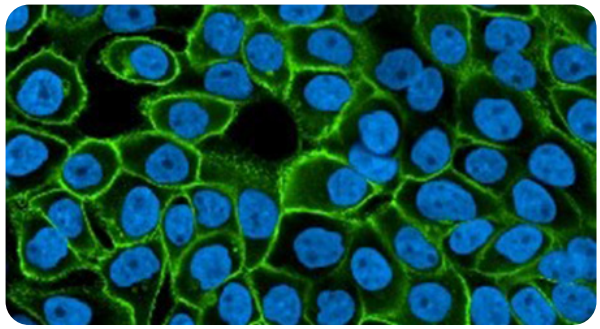
Safety information

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

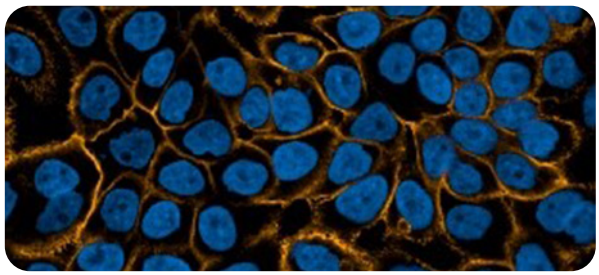
- High-content analysis / high-content screening
- Imaging microscopy
- Flow cytometry

Validation data

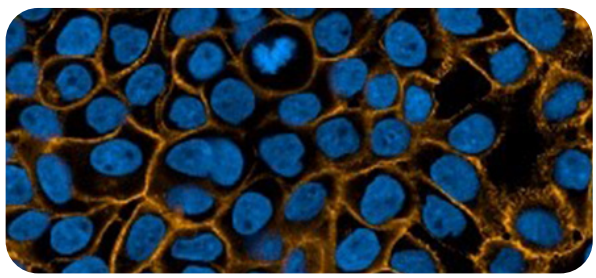


PhenoVue Fluor 488 - Donkey anti-Mouse IgG (H+L) Cross-adsorbed

Figure 1: A431 cells were seeded in PhenoPlate 96-well (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 7.5 µg/mL of PhenoVue Fluor 488 – Donkey 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.

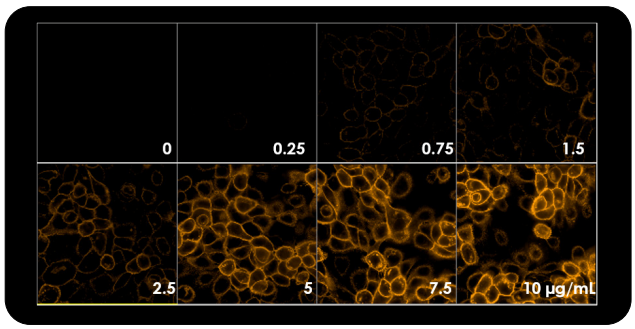


PhenoVue Fluor 555 - Donkey anti-Mouse IgG (H+L) Cross-adsorbed

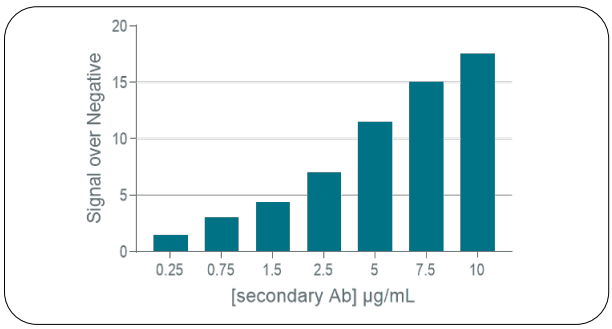


PhenoVue Fluor 555 -Donkey anti-Mouse IgG (H+L) Highly Cross-adsorbed

Figure 2: A431 cells were seeded in PhenoPlate 96-well (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 (0.2 µg/mL). After washing steps, cells were incubated with 5 µg/mL of PhenoVue Fluor 555 - Donkey 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.



PhenoVue Fluor 568 - Donkey anti-Mouse IgG (H+L) Cross-adsorbed



PhenoVue Fluor 568 Donkey anti-Mouse IgG (H+L) Cross-adsorbed

Figure 3: A431 cells were seeded in PhenoPlate 96-well (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 (1 µg/mL). After washing steps, cells were incubated with increasing concentrations of PhenoVue Fluor 568 - Donkey anti-Mouse IgG (H+L) cross-adsorbed for 1 hour at RT. Images were acquired on the Operetta CLS high-content analysis system.

PhenoVue Fluor 594 - Donkey anti-Mouse IgG (H+L) Highly Cross-adsorbed

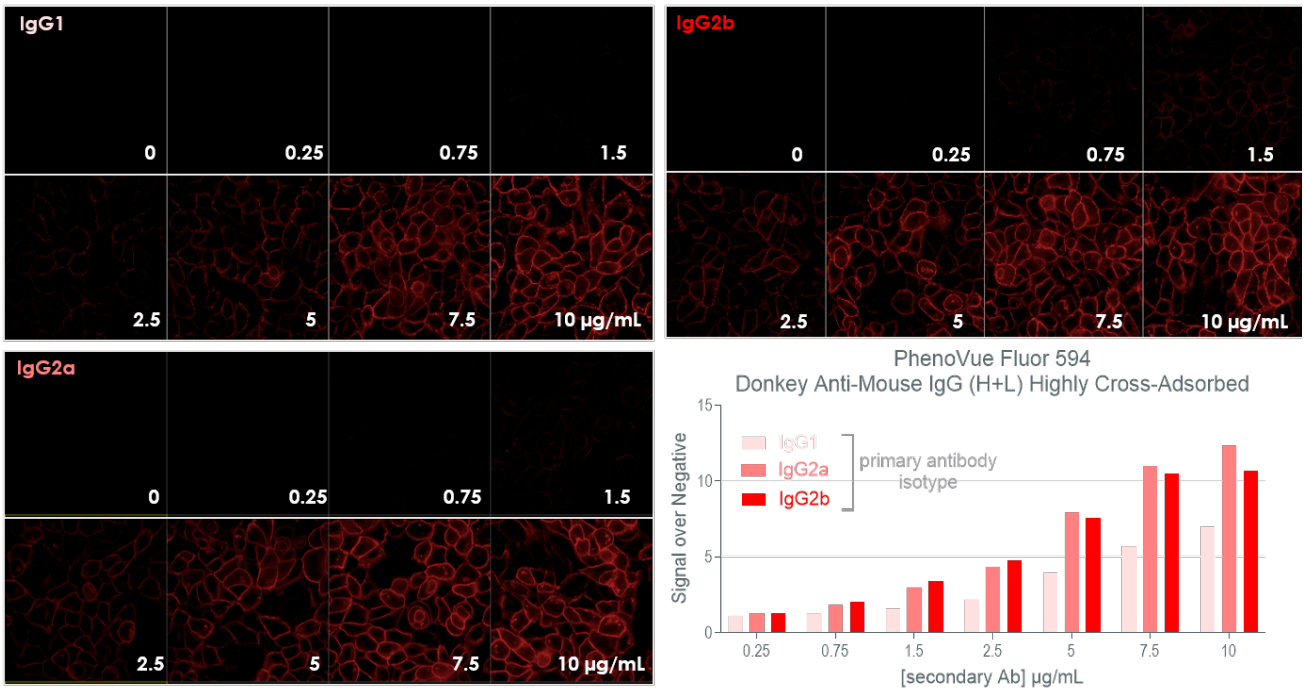
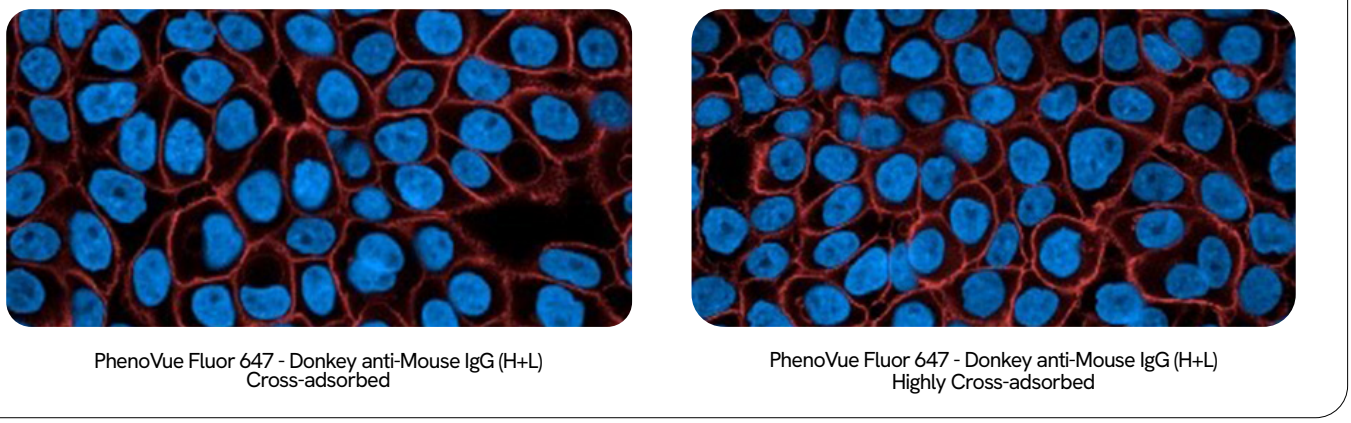


Figure 4: A431 cells were seeded in PhenoPlate 96-well (75,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Cells were fixed then permeabilized and incubated with an anti-EGFR Mouse antibody (1 µg/mL). After washing steps, cells were incubated with increasing concentrations of PhenoVue Fluor 594 - Donkey anti-Mouse IgG (H+L) Highly Cross-adsorbed for 1 hour at RT. Images were acquired on the Operetta CLS high-content analysis system.



PhenoVue Fluor 647 - Donkey anti-Mouse IgG (H+L) Cross-adsorbed

PhenoVue Fluor 647 - Donkey anti-Mouse IgG (H+L) Highly Cross-adsorbed

Figure 5: A431 cells were seeded in PhenoPlate 96-well (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 (0.2 µg/mL). After washing steps, cells were incubated with 10 µg/mL of PhenoVue Fluor 647 - Donkey 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.

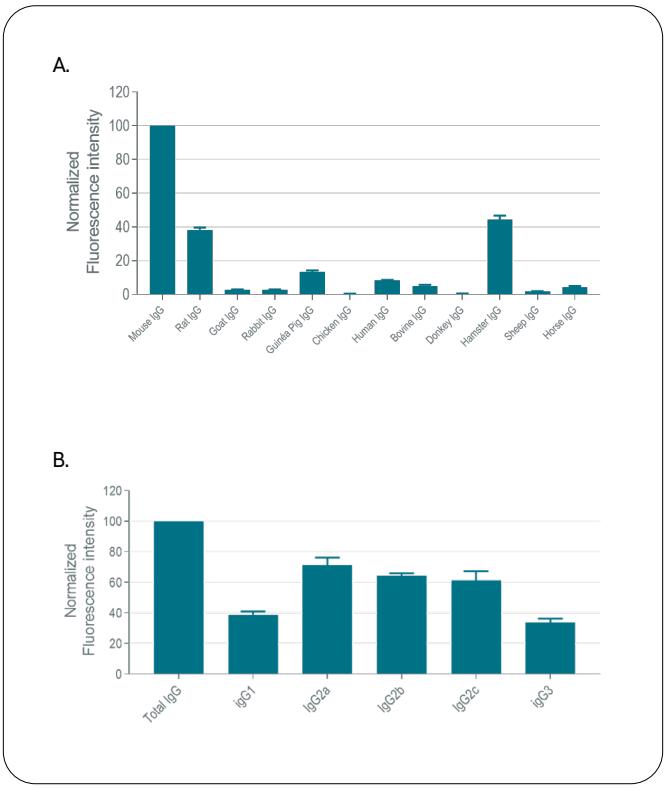


Figure 6.1: F-LISA experiments: different IgG species (A), or IgG isotypes (B) were used to coat a 96-well microplate, then incubated with PhenoVue Fluor 647 - Donkey anti-Mouse IgG (H+L) Cross-Adsorbed (1.5 µg/mL). Fluorescence intensity was measured on an EnVision® multimode plate reader.

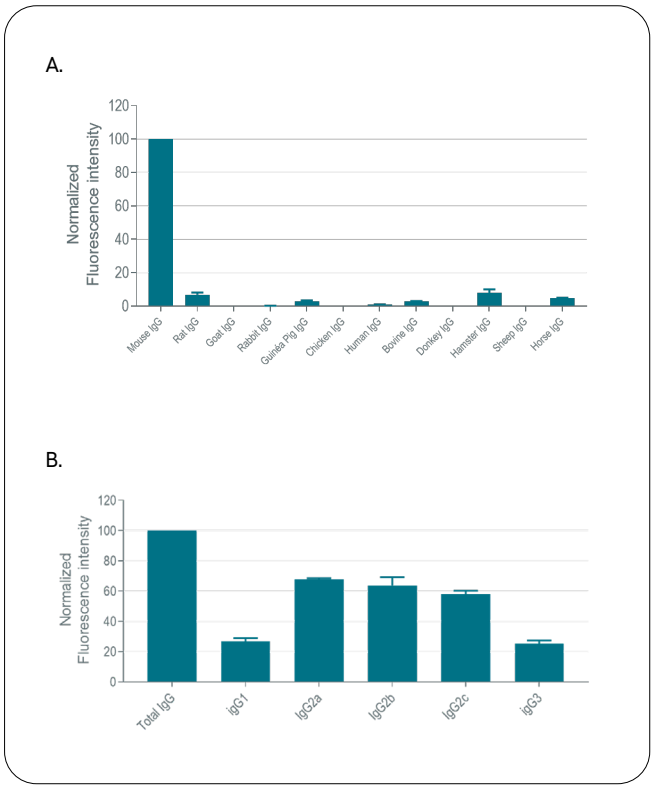


Figure 6.2: F-LISA experiments: different IgG species (A), or IgG isotypes (B) were used to coat a 96-well microplate, then incubated with PhenoVue Fluor 647 - Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed (1.5 µg/mL). Fluorescence intensity was measured on an EnVision multimode plate reader.

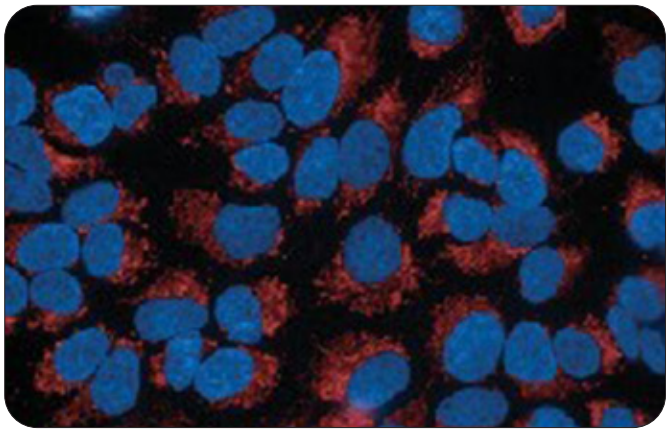


Figure 7: HeLa cells were seeded in PhenoPlate 96-well (50,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. Live cells were stained with 150 nM of PhenoVue 641 Mitochondrial stain for 30 min at 37 °C prior to fixation and permeabilization. Images were acquired on the Operetta CLS high-content analysis system.

Figure 8: PhenoPlate 96-well microplate

Related products

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Operetta® CLS™ High-Content Analysis System

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