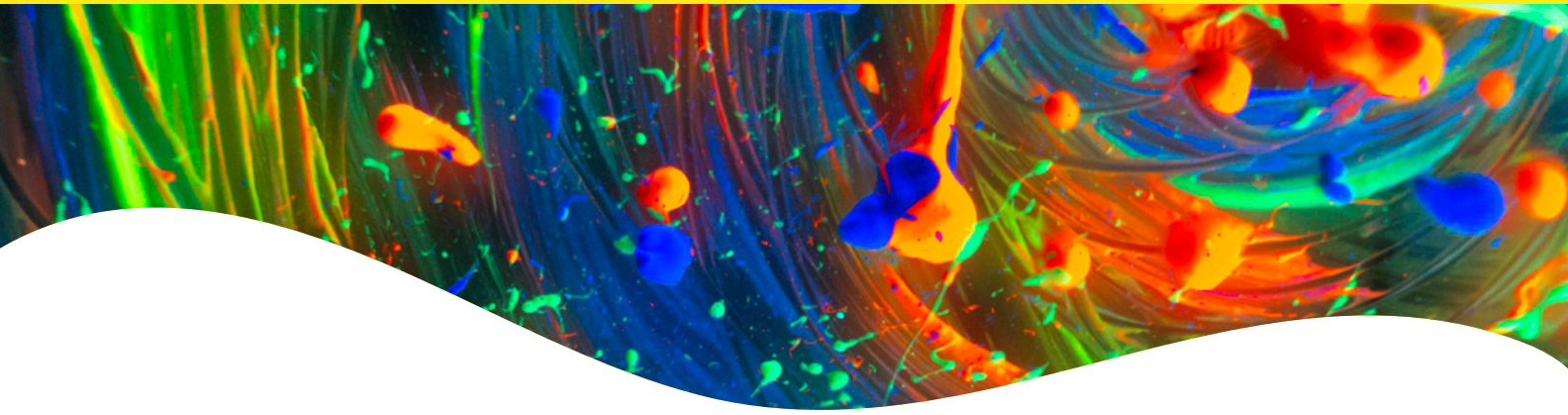
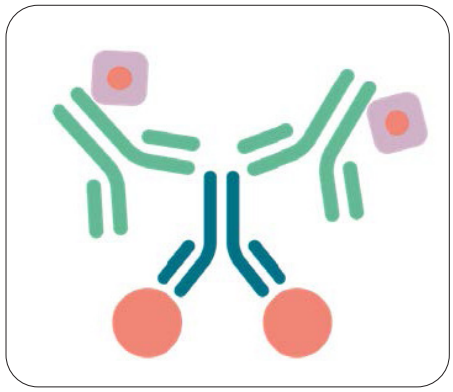
PhenoVue Fluor - Rat anti-Mouse IgG Isotype Specific Antibody Conjugates



Overview

Rat anti-mouse IgG isotype specific monoclonal antibodies are conjugated with our bright PhenoVue™ Fluor dyes.

PhenoVue Fluor - Rat anti-mouse IgG isotype specific antibodies, highly cross-adsorbed have been adsorbed against various IgG isotypes as well as 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 - Rat anti-mouse IgG1 highly cross-adsorbed	2RTXM488G1H1	1	0.5 mg	Lyophilized	RT
PhenoVue Fluor 647 - Rat anti-mouse IgG2a highly cross-adsorbed	2RTXM647G2AH1				

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 - Rat anti-mouse IgG1 highly cross-adsorbed	150000 g/mol	Reconstitution using 0.5 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 647 - Rat anti-mouse IgG2a 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 - Rat anti-mouse IgG1 highly cross-adsorbed	2.5 µg/mL (20 nM)	Approx. 6-20	Approx. 6-20	Approx. 11-32
PhenoVue Fluor 647 - Rat anti-mouse IgG2a 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 647	650	670	Cy5	30%	240000	72000

* In methanol

Cross-reactivity

Product name	Across species	Across IgG isotypes
PhenoVue Fluor 488 - Rat anti-mouse IgG1 highly cross-adsorbed	Mouse specific	IgG1 specific
PhenoVue Fluor 647 - Rat anti-mouse IgG2a highly cross-adsorbed	Mouse specific	IgG2a specific

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:
   - 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

   - Add 100% methanol (chilled to -20 °C) at room temperature for 5 min.
2. **Staining:** Incubate with 0.1-10 µg/mL PhenoVue Fluor - Rat anti-mouse IgG isotype specific antibody highly cross-adsorbed for 60 min at RT.
3. **Washing:** Wash three times with PBS for 5 min.
4. **Optional:** Incubate with 0.1-2 µg/mL PhenoVue Hoechst 33342 nuclear stain for 10 min.
5. **Washing:** Wash once with PBS for 5 min.
6. **Acquire** images on an imaging device.

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

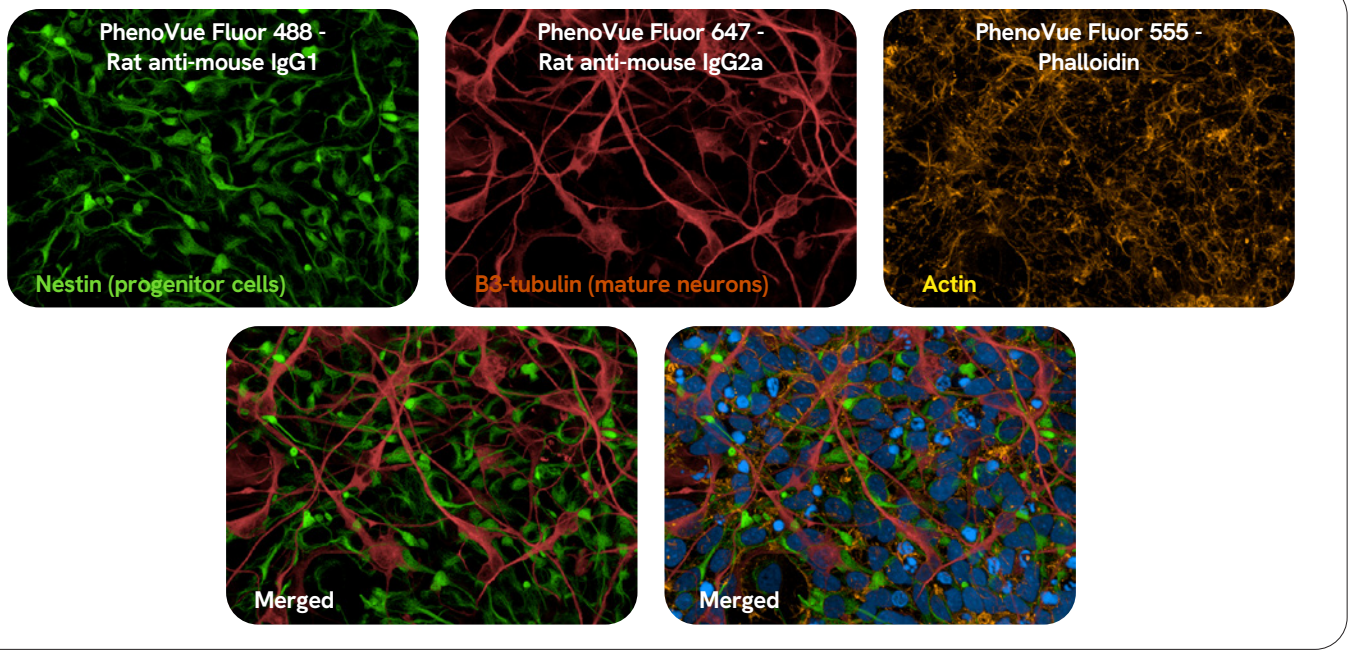


Figure 1: iPSC-derived human cortical neurons were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2. 7 days post-seeding, cells were fixed, permeabilized, saturated and stained using the PhenoVue neuronal differentiation staining kit (PNDIF11). Images were acquired on the Operetta CLS (8 LED) high-content analysis system with the 63X water objective.

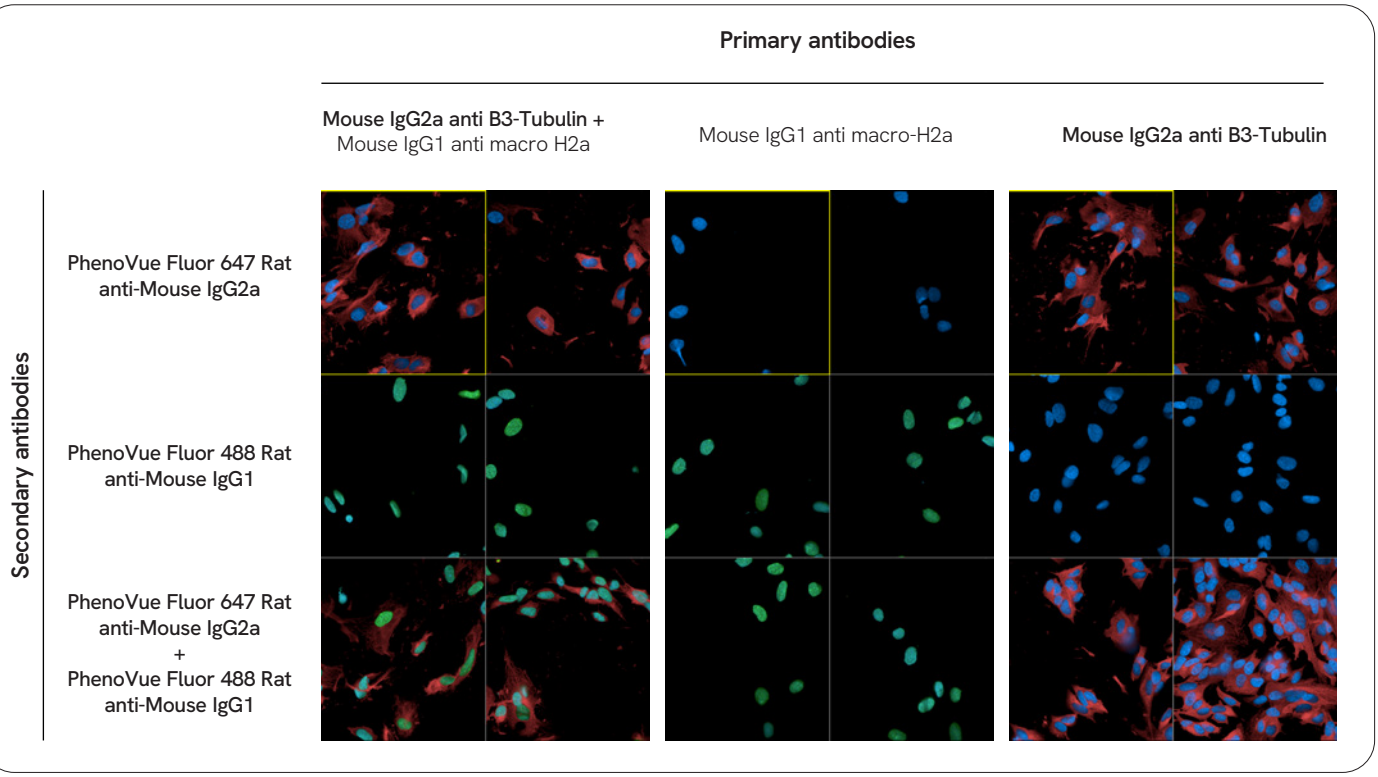


Figure 2: Neuroblastoma SHSY5Y cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. After 24h, cells were fixed, permeabilized, saturated and incubated overnight with PhenoVue anti-B3 tubulin mouse IgG2a, anti-mouse macro H2 IgG1 either alone or mixed. Cells were then stained with the PhenoVue Rat anti-mouse IgG1 and IgG2a. Images were acquired on the Operetta CLS (8 LED) high-content analysis system with the 63X water objective and the 63X water objective. These results demonstrate the isotype specificity recognition obtained with of PhenoVue Fluor - Rat anti-mouse IgG1 and IgG2a.

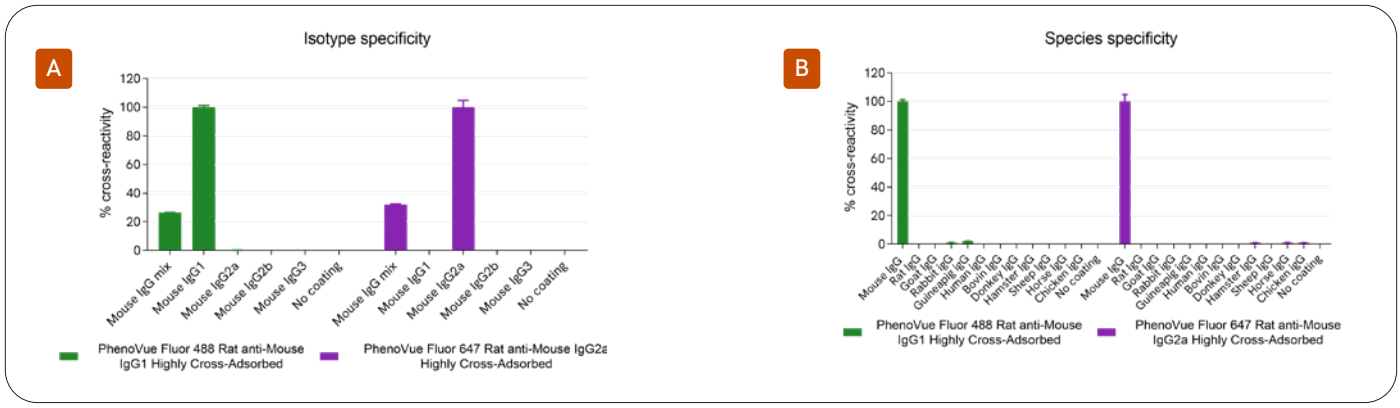


Figure 3: F-LISA experiments: different IgG isotypes (A) or rat IgG species (B) were used to coat a 96-well microplate, then incubated with PhenoVue Fluor 488 – Rat anti-mouse IgG1 and PhenoVue Fluor 647 – Rat anti-mouse IgG2a (5 µg/mL). Fluorescence intensity was measured on an Envision multimode plate reader. These results confirm the high specificity of these two secondary antibodies.

Figure 4: PhenoPlate 96-well microplate.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com