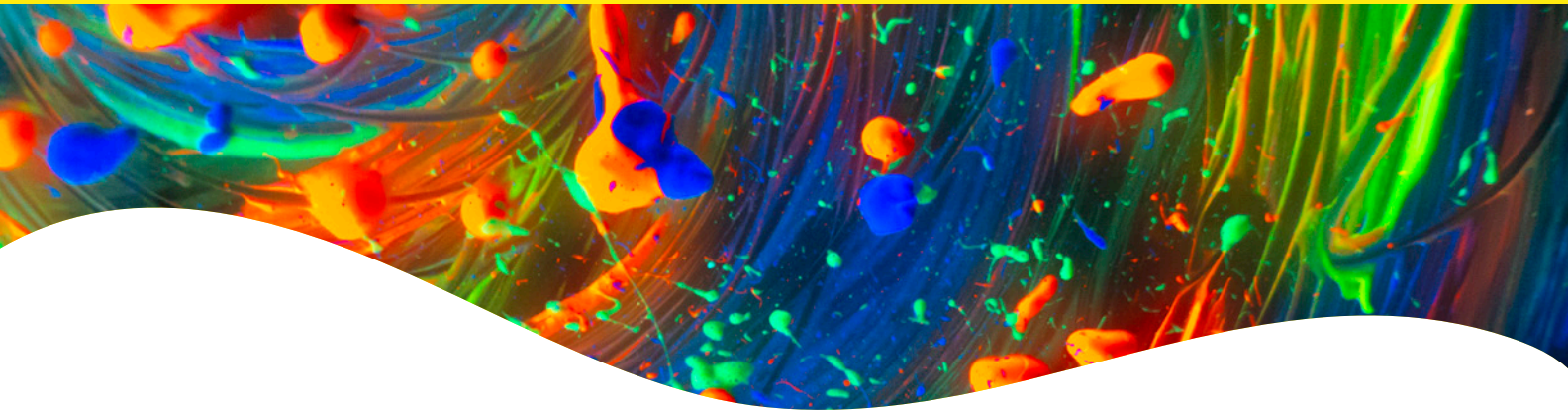
PhenoVue anti-B3 Tubulin Antibody



Overview

B3-tubulin is one of the seven β-tubulin isotypes predominantly expressed in neurons and widely used as a marker to distinguish neurons from other cell types. B3-tubulin is involved in neurogenesis, axon guidance and maintenance. Its expression increases with neuronal maturation and is maintained at latest maturation stages.

PhenoVue™ anti-B3-tubulin antibody is part of the PhenoVue neuronal differentiation staining kit (part number: PNDIF11) and can be used as an individual reagent in imaging and broader high-content analysis applications.

Product information

Product name	PhenoVue anti-B3 tubulin antibody
Part number	PAB3TUB1
Packaging	1 vial of 100 µL
Concentration	100X
Format	Liquid
Clonality	Monoclonal
Host species	Mouse
Isotype	IgG2a,k
Cross-reactivity	Human, mouse and rat
Immunogen	This antibody was raised against microtubules derived from rat brain
Purification	Affinity chromatography
Formulation	PO_4 pH7 100mM -0.1% BSA
Applications	High content analysis (immunofluorescence, imaging, microscopy)
Shipping conditions	Dry ice
Storage conditions	-16°C or below. Protect from light

Stability

- The stability of this product is guaranteed until the expiration date provided in the Certificate of Analysis, when stored as recommended and protected from light.
- To avoid multiple freeze/thaw cycles, freeze the stock solution in aliquots.

Protocols

Cell culture

Seed cells in PhenoPlate 384-well 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:** Add ready to use PhenoVue paraformaldehyde 4% methanol-free solution (PVPFA41) for 10-20 min at room temperature.
2. **Washing:** Wash three times with PBS for 5 min.
3. **Permeabilization:** Add PhenoVue permeabilization solution diluted at 0.1% Triton X-100 in PBS, for 10 min at room temperature.
4. **Washing:** Wash three times with PBS for 5 min.
5. **Saturation:** Incubate with PBS-1% BSA for 1h at room temperature.
6. **Washing:** Wash three times with PBS for 5 min.
7. **Primary antibody incubation:** Add 25 µL per well of mouse monoclonal anti-LAMP1 antibody and incubate for 1-3h at room temperature or overnight at 4 °C.
8. **Washing:** Wash three with PBS for 5 min.
9. **Fluorescent secondary antibody incubation:** Add 25 µL per well of PhenoVue Fluor goat anti-rat antibody and incubate for 1h at room temperature, protected from light.
10. **Washing:** Wash three times with PBS for 5 min.
11. **Acquire images on an imaging device.**

* PhenoPlate 96-well microplates may also be used. Adjust the cell density accordingly and increase the corresponding volumes by 2-fold.

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

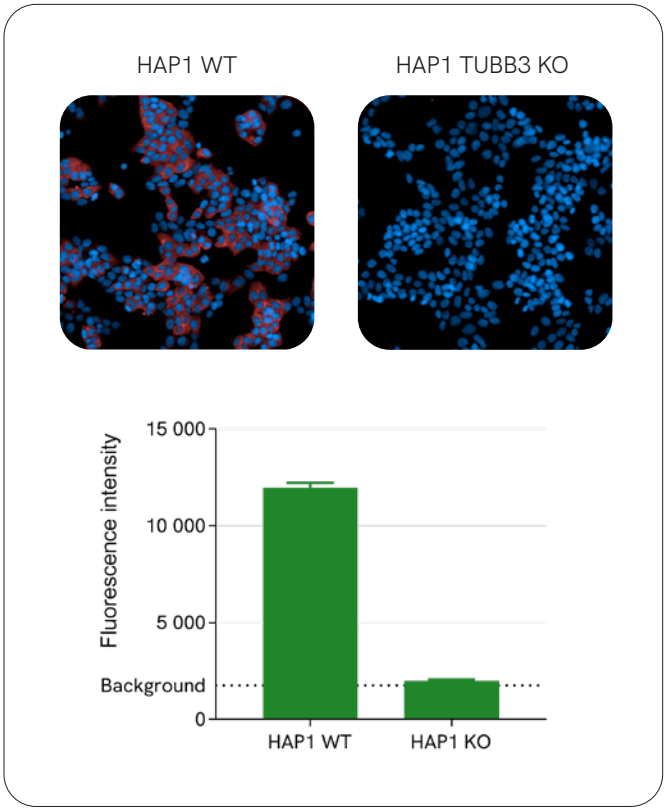


Figure 1: HAP1 WT and HAP1 TUBB3 KO were stained with PhenoVue anti-B3 tubulin antibody and PhenoVue Fluor 647 - Goat anti-mouse highly cross adsorbed antibody. As expected, no fluorescent signal was detected in the TUBB3 KO cells, validating the specificity of PhenoVue anti-B3 tubulin antibody.

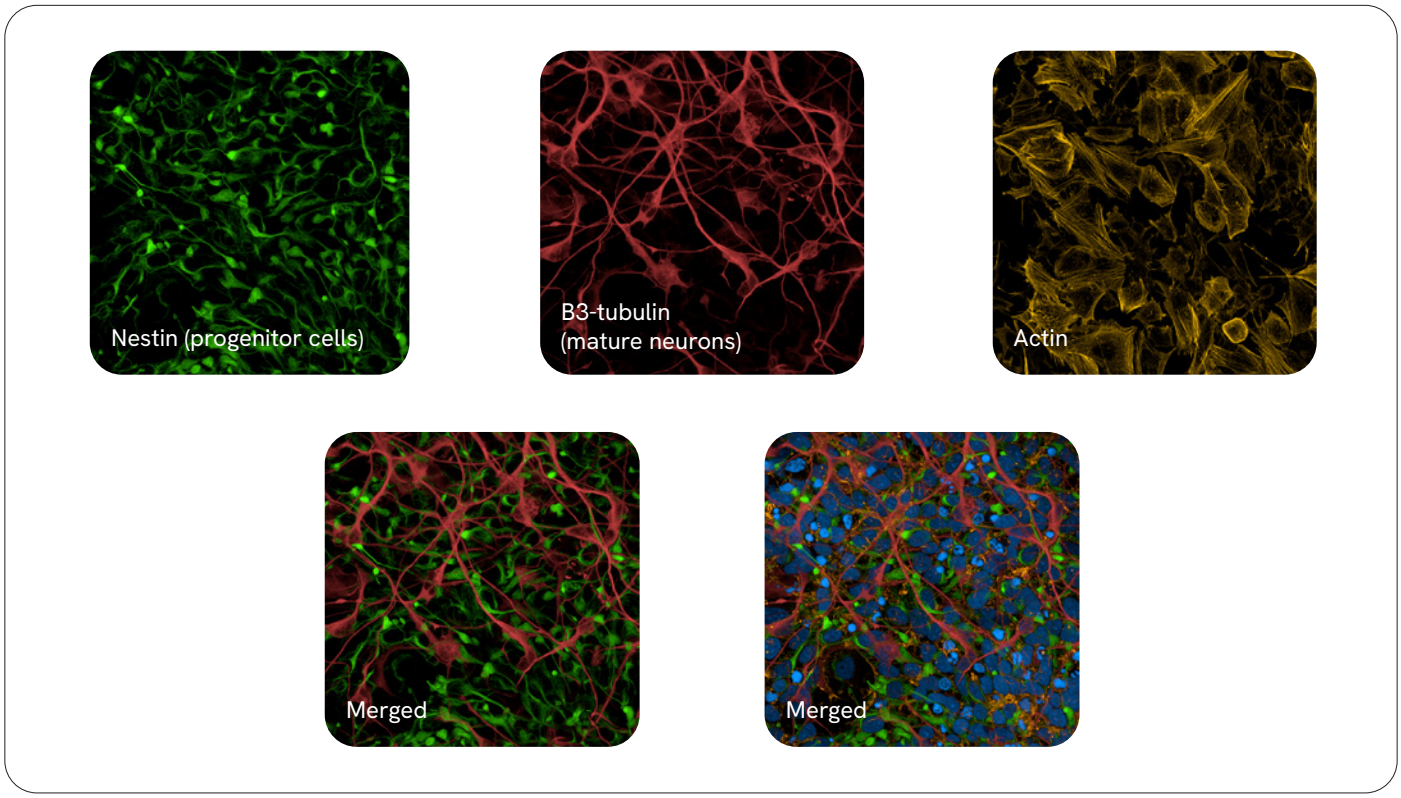


Figure 2: IPS-derived human cortical neurons were seeded in PhenoPlate 96-well microplates (40,000 cells/well) and incubated at 37 °C, 5% CO_2. Seven days post-seeding, cells were fixed, permeabilized, saturated and stained using the PhenoVue neuronal differentiation staining kit (part number: PNDIF11). Images were acquired on the Operetta CLS (8 LED) high-content analysis system with the 63X water objective.

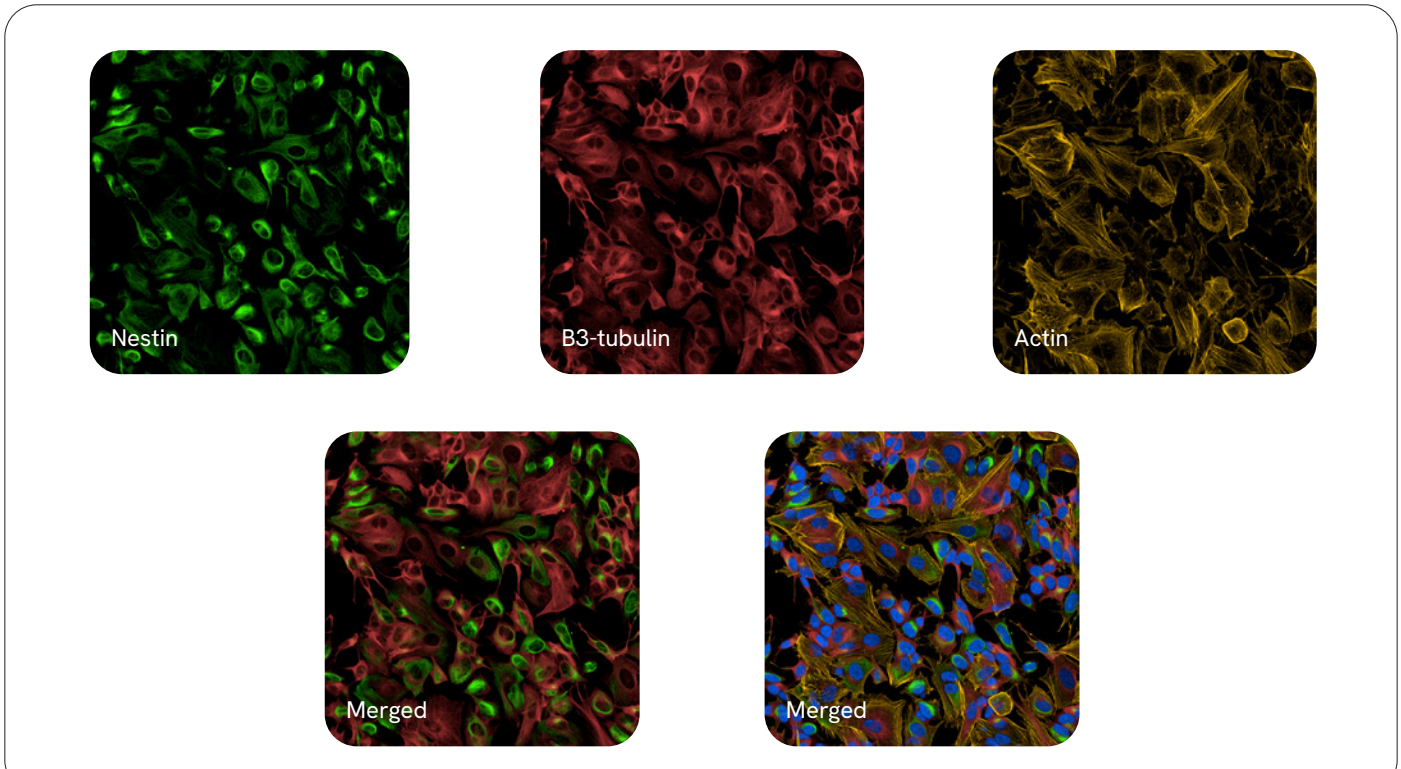


Figure 3: Neuroblastoma SHSY5Y cells were seeded in PhenoPlate 96-well microplates (75,000 cells/well) and incubated at 37 °C, 5% CO_2 for 24h. After 24h, cells were fixed, permeabilized, saturated and stained using the PhenoVue neuronal differentiation staining kit (part number: PNDIF11). Images were acquired on the Opera Phenix Plus (5 lasers) high-content screening system with the 63X water objective.

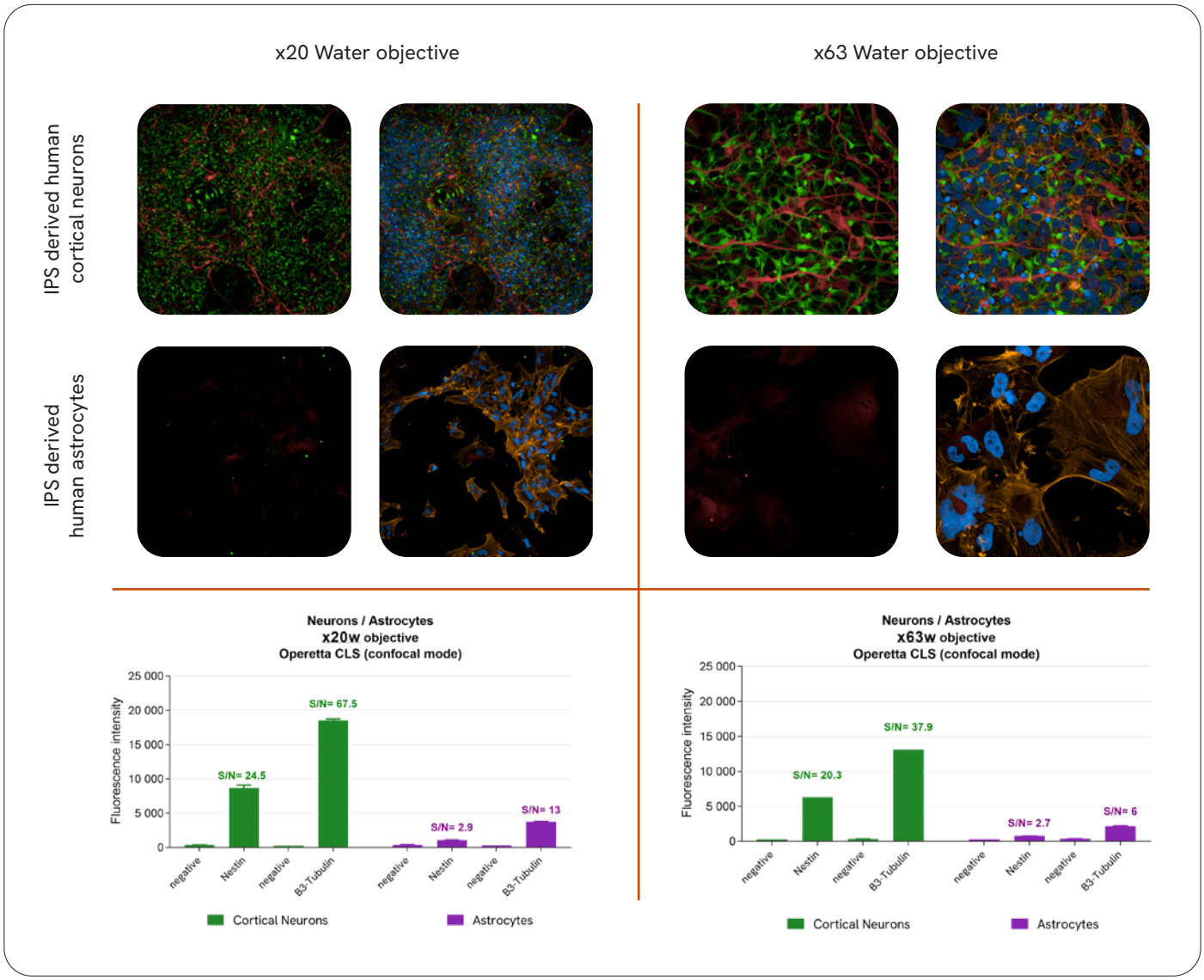


Figure 4: IPS-derived human cortical neurons (40,000 cells/well) or IPS-derived human astrocytes (30,000 cells/well) were seeded in PhenoPlate 96-well microplates and incubated at 37 °C, 5% CO_2. Respectively, seven or four days post-seeding, cells were fixed, permeabilized, saturated and stained using the PhenoVue neuronal differentiation staining kit (part number: PNDIF11); primary antibodies were incubated 3h at RT). Images were acquired on the Operetta CLS (8 LED) high-content analysis system with the 20X water objective and the 63X water objective.

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