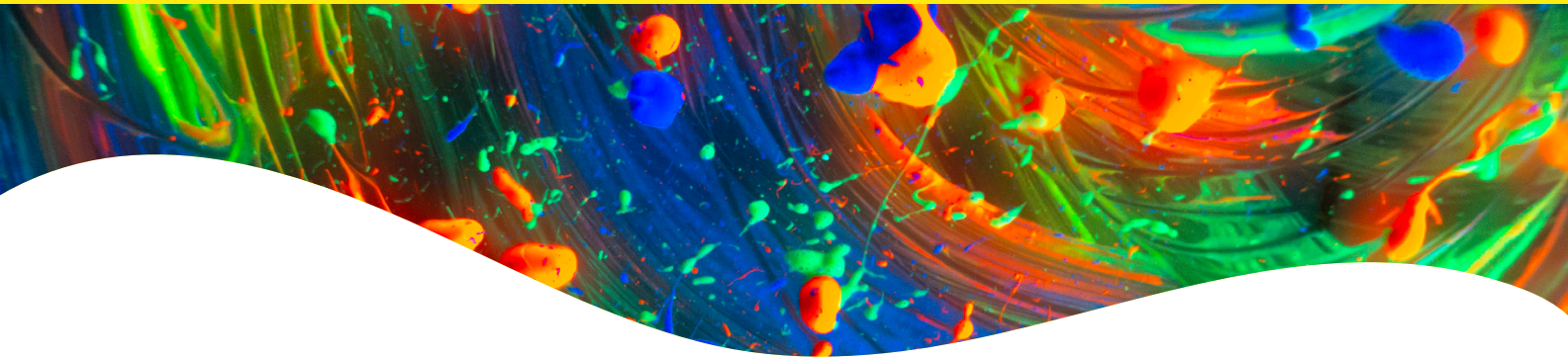
PhenoVue Astrocyte Differentiation Staining Kit (for 1 x 384-well plate)



Overview

In neuroscience research, the use of physiologically relevant cell models, such as iPSC-derived neuronal and glial cells, is key to better understanding neurodevelopmental processes and disease mechanisms. Astrocytes, as the most abundant glial cells in the central nervous system, play critical roles in synaptic support, blood-brain barrier maintenance, and neuroinflammatory responses.

Given the complexity of astrocyte differentiation and the importance of validating astrocyte identity, the PhenoVue™ Astrocyte differentiation staining kit offers a robust solution for confirming astrocyte phenotype through a multiplexed staining approach.

This ready-to-use kit enables the visualization and qualification of iPSC-derived astrocytes by combining a well-characterized antibody against GFAP (glial fibrillary acidic protein), the gold-standard astrocyte marker, with actin cytoskeleton and nuclear stains to assess cell morphology and organization.

The PhenoVue Astrocyte differentiation staining kit includes three fluorescent markers:

- **GFAP:** Glial fibrillary acidic protein is an intermediate filament protein highly expressed in mature astrocytes and widely used to distinguish astrocytes from other CNS cell types.
- **Phalloidin** (conjugated to PhenoVue Fluor 555): provides detailed cytoskeletal visualization for morphology assessment.
- **PhenoVue Hoechst 33342:** nuclei staining for cell identification and quantification.

Each component of the kit has been extensively validated and optimized, ensuring maximum spectral separation with no overlap, even when adding additional fluorescent stains, such as those compatible with the PhenoVue Fluor 647 channel, to measure other proteins or cellular events in parallel. Furthermore, the specificity of each reagent has been rigorously confirmed, making this kit an easy-to-use solution for qualifying astrocyte differentiation and assessing activation states based on cellular morphology.

Product information

Product name	Part no.	Number of vials per unit	Shipping conditions
PhenoVue Astrocyte differentiation staining kit – 1 x 384 wells	PADIF11	7	Dry ice

Product name	Format	Quantity*	Storage
PhenoVue Hoechst 33342 nuclear stain	Liquid (H_2O)	1 vial of 70 µL (500x)	2-8 °C or below. Protect from light
PhenoVue Fluor 555 - Phalloidin (600x)	Desiccated	1 vial of 60 µL (600x)	-16 °C or below. Protect from light
PhenoVue anti-GFAP mouse IgG1 antibody (100x)	Liquid	1 vial of 100 µL (100x)	-16 °C or below
PhenoVue Fluor 488 - Goat anti-mouse antibody, highly cross-adsorbed (100x)	Liquid	1 vial of 100 µL (100x)	-16 °C or below. Protect from light
PhenoVue Paraformaldehyde, 4% solution	Liquid	1 vial of 25 mL (1x)	2-8 °C or below. Protect from light
PhenoVue Permeabilization, 0.5% Triton X-100 solution	Liquid	1 vial of 25 mL (5x)	2-8 °C or below
PhenoVue Dye diluent A (5x)	Liquid	1 vial of 8 mL (5x)	-16 °C or below

*The quantities of reagents provided are sufficient for 2 x 96 or 1 x 384-well microplate using the recommended concentrations.

Storage and stability

- After receiving, store PhenoVue Permeabilization 0.5% Triton X-100 solution and PhenoVue Paraformaldehyde, 4% solution at 2–8 °C, protected from light.Other reagents can be stored together at ≤ -16 °C or separately between ≤ -16 °C to 2-8 °C protected from light, as indicated in the table above.
- Allow the reagents to warm up to room temperature for 30 min before opening the vials and reconstitution. Aliquoted reagents must be stored at -16 °C or below and are stable for 6 months.
- After thawing, the PhenoVue dye diluent A (5x) may contain some aggregates which will not impair the product and image quality. If aggregate removal is preferred, the PhenoVue Dye diluent A (5x) can be filtered (0.22 µm filter) prior to dilution. The diluted PhenoVue Dye diluent A 1x must be stored at 2-8 °C for no more than 2 days.
- 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.

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 Hoechst 33342	357**	455**	DAPI	dsDNA: 0.38 ssDNA: 0.22	43,000	dsDNA: 16,340 ssDNA: 9,460
PhenoVue Fluor 488	495	520	FITC	92%	73,000	65,320
PhenoVue Fluor 555	555	570	Cy3	10%	155,000	15,500

*In PBS pH 7.4 ** In methanol with 0.2 M HCl

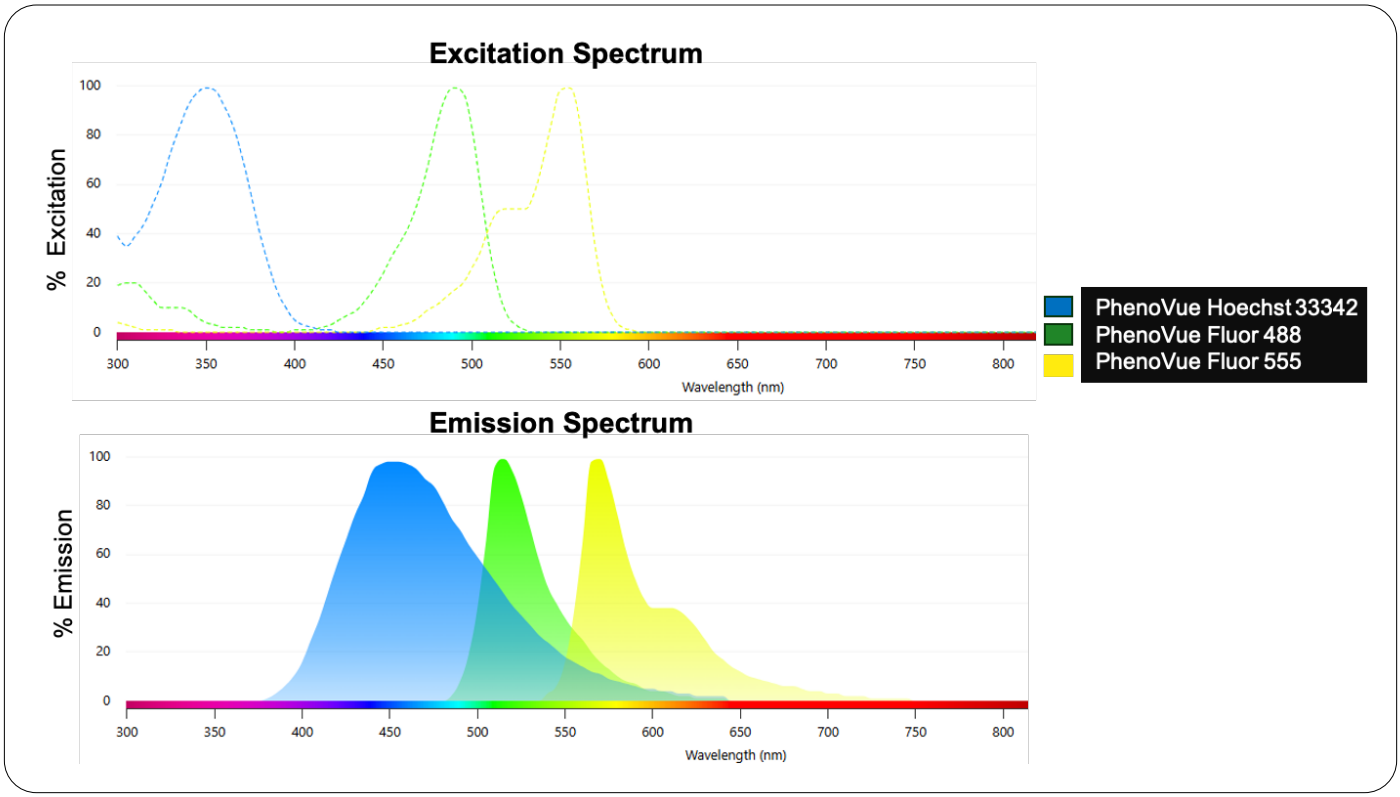


Figure 1: Excitation and emission spectra for the PhenoVue Astrocyte differentiation staining kit components.

Other materials and reagents required

Reagents or consumables	Usage
PBS	Washing buffer and diluent for PhenoVue Permeabilization 0.5% Triton X-100 solution
Distilled H_2O	Dilution of PhenoVue Dye diluent A (5x)
PhenoPlate™ 384-well microplates*	Cell plating, stimulation, staining and imaging
Aluminum single-tab foil	Plate sealing to protect fluorescent probes from light

*This protocol can be adapted for use with PhenoPlate 96-well microplates. See Protocol Section for details.

Reagent reconstitution and preparation of staining solutions

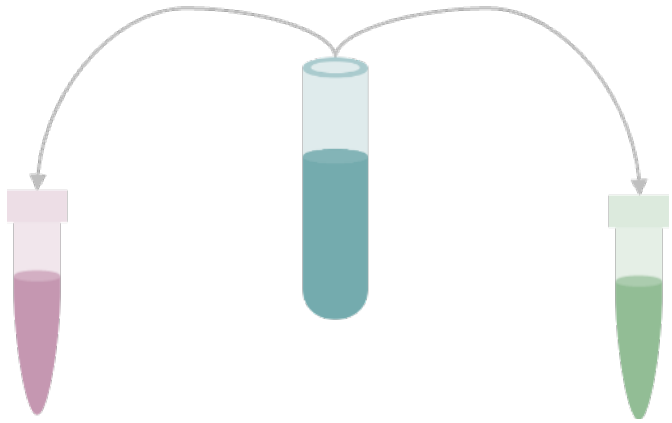
	Reagents	Preparation of staining solution
Buffers	PhenoVue Paraformaldehyde, 4% solution	Ready to use
	PhenoVue Permeabilization 0.5% Triton X-100 solution	Dilute 5 times in PBS
	PhenoVue Dye diluent A (5x)*	Dilute 5 times in distilled H_2O
Staining solution 1	PhenoVue anti-GFAP mouse antibody (100x) Human only	Dilute 100 times in diluent A (1x)
Staining solution 2	PhenoVue Hoechst 33342 Nuclear stain	Dilute 500 times in diluent A (1x)
	PhenoVue Fluor 555 – Phalloidin (600x)	Reconstitute with 60 µL DMSO and dilute 600 times in diluent A (1x)
	PhenoVue Fluor 488 - Goat anti-mouse antibody, highly cross-adsorbed (100x)	Dilute 100 times in diluent A (1x)

* PhenoVue Dye diluent A 1x is used as staining solution diluent and as saturation buffer.

Example preparation of staining solutions

The following example describes the preparation of 10 mL staining solution 1 and 10 mL of staining solution 2, sufficient for 1 x 384-well plate or 2 x 96-well plates.

Prepare **20 mL PhenoVue Dye diluent A (1x)**
To 16 mL ddH_2O, add 4 mL PhenoVue Dye diluent A (5x)



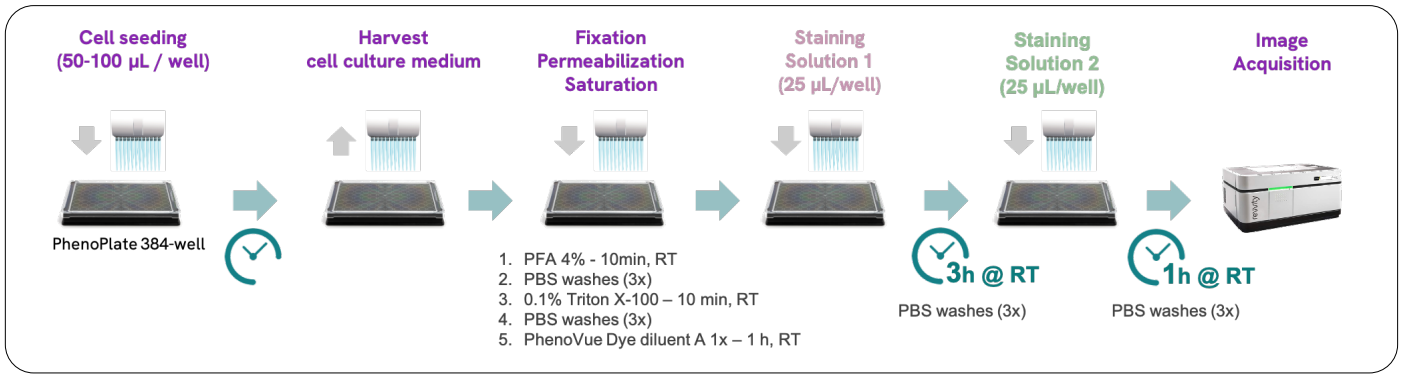
10 mL staining solution 1
To 9.9 mL PhenoVue Dye diluent (1x)

- Add 100 µL of PhenoVue anti-GFAP – mouse antibody (100x)

10 mL staining solution 2
To 9.864 mL PhenoVue Dye diluent A (1x)

- Add 20 µL of PhenoVue Hoechst 33342 Nuclear stain (500x)
- Add 16.7 µL of PhenoVue Fluor 555 – Phalloidin (600x)
- Add 100 µL PhenoVue Fluor 488 - goat anti-mouse antibody highly cross-adsorbed (100x)

Experimental workflow



Protocol for 384-well imaging plate

A 96-well microplate may also be used; in that case, adjust the cell seeding density appropriately and increase all reagent volumes two-fold.

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 25 µL ready-to-use PhenoVue paraformaldehyde 4% solution for 10 min at room temperature.
2. **Washing:** Wash three times with PBS.
3. **Permeabilization:** Add 25 µL PhenoVue permeabilization solution diluted to 0.1% Triton X-100 in PBS for 10 min at room temperature.
4. **Washing:** Wash three times for 5 min with PBS.
5. **Saturation:** Incubate with 25 µL PhenoVue dye diluent A 1x for 1 h at room temperature.
6. Remove saturation buffer and **add 25 µL per well of staining solution 1.** Incubate for 3 h at room temperature.
7. **Washing:** Wash three times for 5 min with PBS.
8. **Add 25 µL per well of staining solution 2** and incubate for 1 h at room temperature protected from light.
9. **Washing:** Wash three times with PBS for 5 min.
10. **Acquire images on an imaging device.**

Tips

- Reagent concentrations have been carefully optimized to limit fluorescence crosstalk on Revvity's high content screening instruments. Thus, increasing the concentrations may increase crosstalk.
- If needed, when using 384-well microplates, centrifuging the plate for 1 min at 500 g and room temperature between each step allows reagents to settle at the bottom of the wells.
- For reconstituting PhenoVue Fluor 555 – Phalloidin, avoid using methanol or other alcohol-based or aqueous solvents. Anhydrous DMSO is preferable, as it preserves the integrity of actin filaments, resulting in brighter staining.
- The PhenoVue anti-GFAP antibody has been validated on human models exclusively.
- Optimal fluorescence intensities are typically achieved after 3 hours of incubation with primary antibodies at room temperature. While overnight incubation at 4 °C is possible, it is not recommended.
- For added flexibility, the PhenoVue Fluor 647 channel is available for detecting additional proteins or events using other compatible fluorescent stains. In such cases, the following points should be considered:
  - The PhenoVue anti-GFAP antibody is Mouse IgG1.
  - The secondary PhenoVue Fluor 488 Goat anti-mouse antibody is highly cross-adsorbed. While it is highly specific to mouse antibodies, it is not isotype-specific and can therefore recognize IgG1, IgG2a, IgG2b, IgG2c, and IgG3.

- A primary antibody can be easily combined with PhenoVue Fluor 647 secondary antibodies, as long as the primary antibody is not of mouse origin. For instance, PhenoVue Fluor 647 goat anti-rat (part number: 2GXRT647H1) or anti-rabbit (part number: 2GXRB647H1), both highly cross-adsorbed, are compatible for such applications.
- A mouse primary antibody of a different isotype from IgG1 can be used. However, in this case, the secondary goat anti-mouse antibody included in the kit must be replaced with a highly specific anti-mouse IgG1 antibody. The secondary antibody used to detect the additional mouse primary antibody must be highly isotype-specific and should not detect mouse IgG1.

Recommendations for acquisition settings

The PhenoVue Astrocyte differentiation staining kit allows for multiplexing with 3 colors simultaneously. For optimal fluorescent signal and image quality on a Revvity high-content screening system, we recommend the following acquisition settings, depending on the instrument used:

HCS instruments		PhenoVue Hoechst 33342	PhenoVue Fluor 488	PhenoVue Fluor 555
Opera Phenix™ Plus 5 lasers	Excitation laser (nm)	375	488	561
	Emission filters (nm)	435-480	500-550	570-630
Opera Phenix Plus 4 lasers	Excitation laser (nm)	405	488	561
	Emission filters (nm)	435-480	500-550	570-630
Operetta CLS 4 or 8 LED	Excitation LED (filters) (nm)	370 (355-385)	475 (460-490)	550 (530-560)
	Emission filters (nm)	430-500	500-550	570-650

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

Assay validation

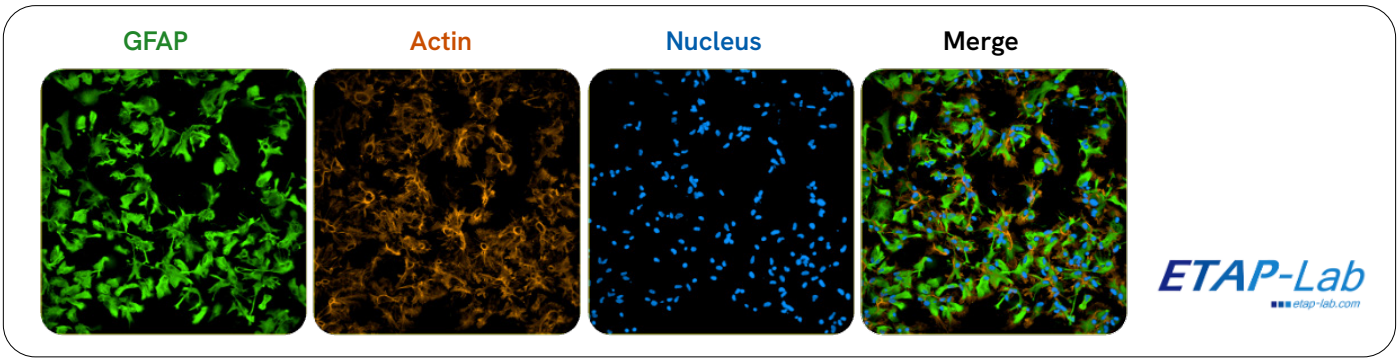


Figure 1: iPSC-derived human astrocytes (ETAP-Lab) labeled using the PhenoVue Astrocyte differentiation staining kit.

Figure 1 shows iPSC-derived human astrocytes seeded in PhenoPlate 96-well microplates and matured for 7 days by ETAP-Lab collaborators. Following fixation with 4% paraformaldehyde (PFA), cells were permeabilized and blocked to prevent non-specific staining. Astrocytes were then labeled using the PhenoVue Astrocyte differentiation staining kit which enables specific detection of astrocytic markers. Images were acquired using the Operetta CLS imaging system equipped with a 20x water-immersion objective in confocal mode.

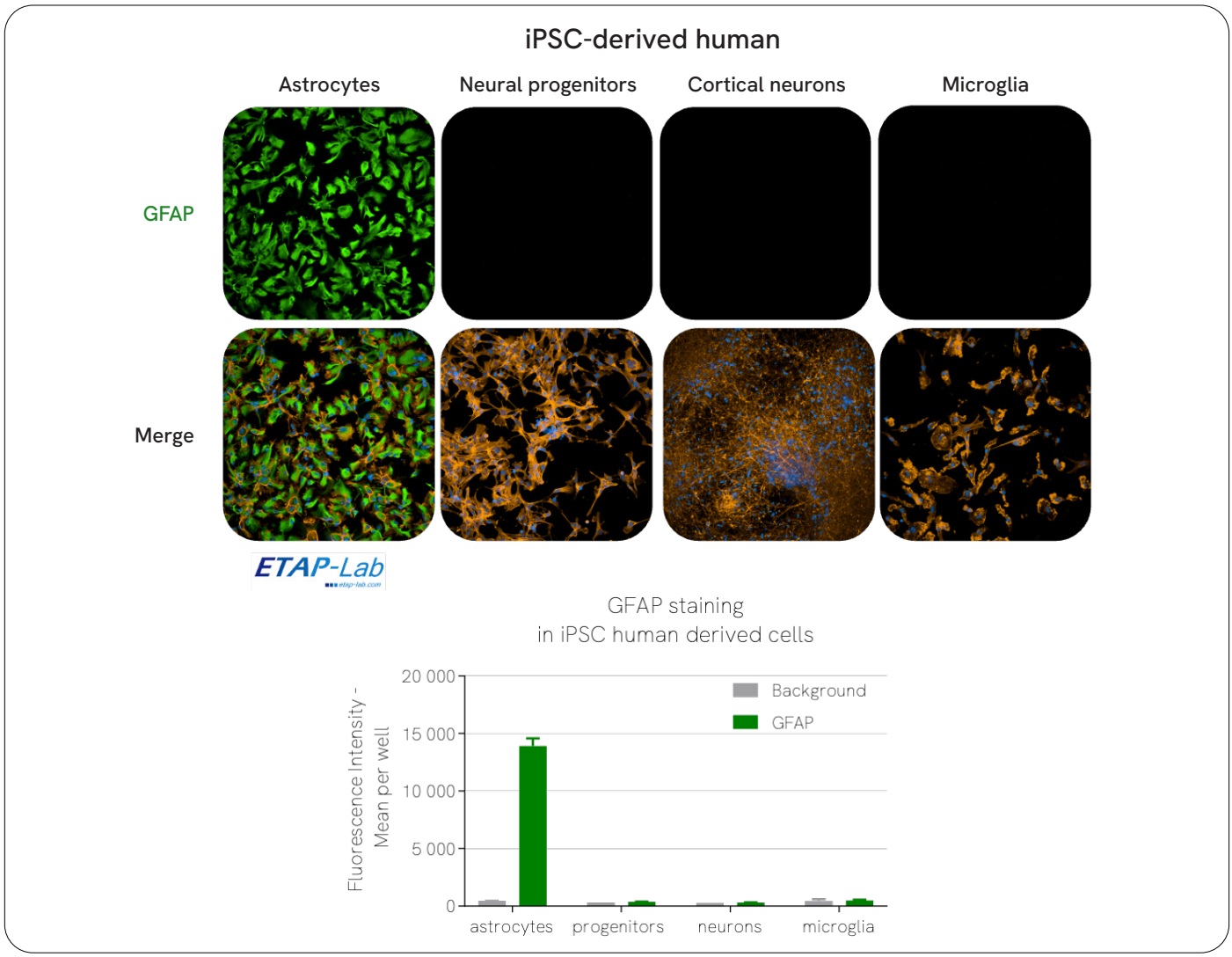


Figure 2: Identification and morphological assessment of various iPSC-derived CNS cell types using the PhenoVue Astrocyte differentiation kit.

Figure 2 shows iPSC-derived human astrocytes (50,000 cells/well, matured for 7 days) from ETAP-Lab, iPSC-derived microglia (10,000 cells/well, 9-day differentiated) from BitBio, iPSC-derived neurons (45,000 cells/well, 30-day differentiated) from INM collaborators in Montpellier (Dr. Coralie Clua Provost and Dr. Carole Crozet, INSERM U1298/Université de Montpellier), and iPSC-derived human neural progenitor cells (30,000 cells/well) from Applied StemCell seeded into PhenoPlate 96-well microplates, following each supplier's recommended protocol.

All cell types were fixed with 4% paraformaldehyde (PFA), permeabilized, blocked to prevent non-specific staining, and stained using the PhenoVue Astrocyte differentiation staining kit. The kit includes an antibody targeting GFAP (green) and a counterstain for actin (orange), providing structural and phenotypic insights. Imaging was performed on the Operetta CLS high-content imaging system using an 8-LED module and a 20x water-immersion objective in confocal mode.

Staining results demonstrate clear phenotypic differences between the cell types: As expected, astrocytes (ETAP-Lab) show strong expression of GFAP (green), confirming efficient astrocytic differentiation. The actin cytoskeleton (orange) reveals a well-spread, stellate morphology typical of mature astrocytes with extensive cell-cell contacts. Neural progenitors exhibit no GFAP expression but prominent actin structures. Microglia show no GFAP expression. Actin staining highlights small, rounded cell bodies with fine, branched processes, indicative of a ramified and resting microglial phenotype. Neurons (INM Montpellier) display dense actin labeling which reveals a highly arborized network of neurites, consistent with mature neuronal morphology and synaptic connectivity. These results demonstrate that the PhenoVue Astrocyte differentiation kit, in combination with high-resolution imaging, enables reliable identification and morphological assessment of various iPSC-derived CNS cell types in a high-throughput format.

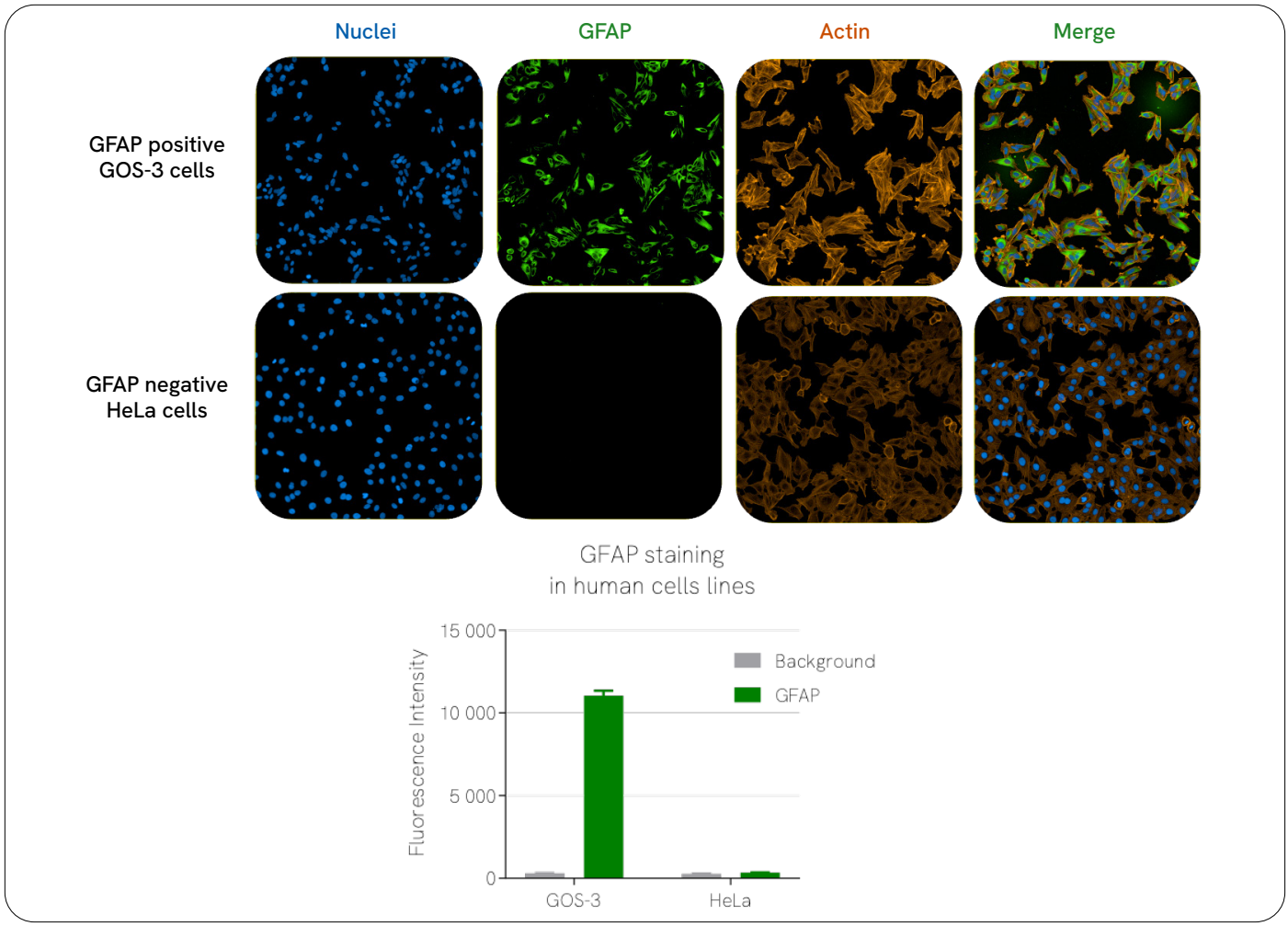


Figure 3: Quantification of GFAP immunostaining confirms the specificity and sensitivity of the PhenoVue anti-GFAP antibody.

Figure 3 shows GOS-3 and HeLa cells seeded in PhenoPlate 96-well microplates at a density of 20,000 cells per well and incubated at 37 °C with 5% CO_2. Following cell attachment, cells were fixed with 4% PFA, permeabilized, saturated, and stained using the PhenoVue Astrocyte differentiation staining kit, following the protocol described above. Primary antibodies were incubated for 3 hours at room temperature.

GOS-3 cells were selected as a GFAP-positive control, as this glioblastoma-derived cell line exhibits high endogenous expression of GFAP (TPM = 4426, according to the Human Protein Atlas). Conversely, HeLa cells were used as a GFAP-negative control, given their very low GFAP expression (TPM = 1.4), making them an appropriate specificity benchmark. Images were acquired using the OperettaCLS high-content imaging system (8-LED configuration) with a 20x water-immersion objective in confocal mode.

Quantification of GFAP immunostaining confirms the specificity and sensitivity of the PhenoVue GFAP antibody. GOS-3 cells, used as a GFAP-positive control, show a strong fluorescence signal (~11,000 AU), significantly above background levels, consistent with their high GFAP mRNA expression. In contrast, no detectable GFAP signal was measured in HeLa cells, which are known to lack GFAP expression.