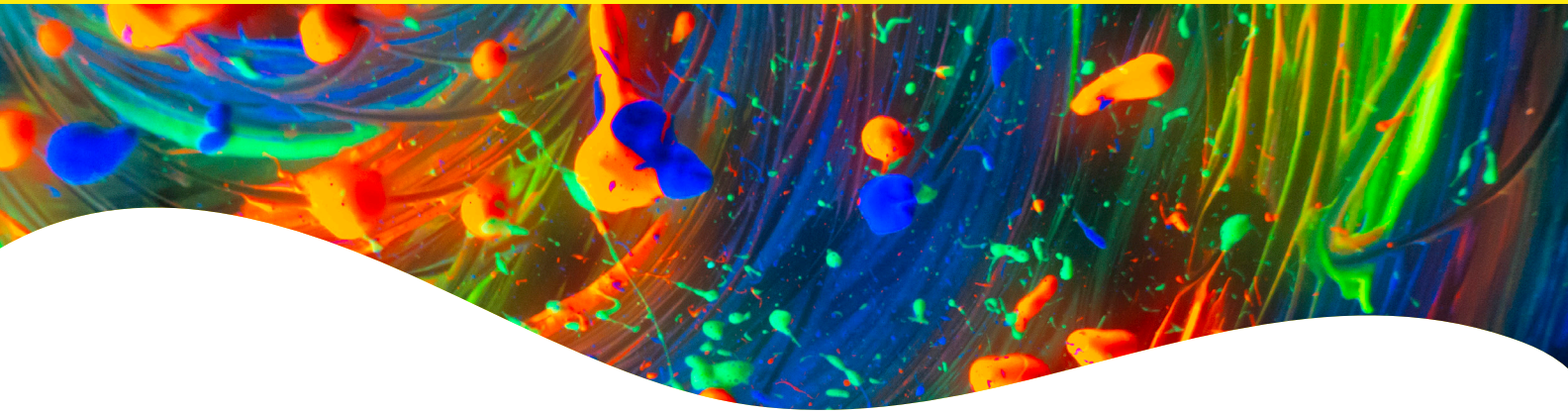
PhenoVue anti-GFAP – mouse IgG1 Antibody



Overview

In neuroscience research, the use of physiologically relevant cell models, such as iPSC-derived neuronal and glial cells, is essential for advancing our understanding of neurodevelopmental processes and disease mechanisms. Among glial cells, astrocytes are the most abundant in the central nervous system (CNS) and play vital roles in synaptic regulation, maintenance of the blood-brain barrier, and neuroinflammatory signaling.

Given the complexity of astrocyte biology and differentiation, accurate identification of astrocytes is critical. The PhenoVue™ anti-GFAP antibody provides a robust and specific tool to confirm astrocyte identity in fixed-cell imaging workflows. GFAP (glial fibrillary acidic protein) is a well-established intermediate filament protein and the gold-standard marker for mature astrocytes, distinguishing them from other central nervous system cell types.

This well-characterized, fluorescence-conjugated antibody enables the clear visualization of GFAP-expressing astrocytes in complex iPSC-derived cultures or primary samples. It is optimized for high-content imaging applications and offers excellent signal-to-noise ratio with minimal background. When combined with additional cellular markers, such as actin stains for morphology or nuclear dyes for quantification, as provided in the PhenoVue Astrocyte differentiation staining kit, this antibody supports multiparametric assessment of astrocyte differentiation and activation states.

Product information

Product name	PhenoVue anti-GFAP – mouse IgG1 antibody
Part number	PAGFAP1
Packaging	1 vial of 100 µL
Concentration	100x
Format	Liquid
Clonality	Monoclonal
Host species	Mouse
Isotype	IgG1, κ
Species	Human verified. Dog, non-human primate predicted.
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Purification	Affinity chromatography
Formulation	PO_4 pH7 100 mM – 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% CO2 until 50-70% confluency.

Fixed-cell imaging protocol

1. **Rinse:** Briefly rinse the cells with phosphate-buffered saline (PBS) before proceeding with fixation.
2. **Fixation:** Add ready-to-use PhenoVue paraformaldehyde 4% methanol-free solution (PVPFA41) for 10-20 min at room temperature.
3. **Washing:** Wash three times with PBS.
4. **Permeabilization:** Add PhenoVue permeabilization solution (PVPERM051) diluted to 0.1% Triton X-100 in PBS, for 10 min at room temperature.
5. **Washing:** Wash three times with PBS.
6. **Saturation:** Incubate with PhenoVue Dye diluent A (PVDDA1), diluted at 1x in H_2O for 1 h at room temperature to block non-specific binding.
7. **Washing:** Wash three times with PBS.
8. **Primary antibody incubation:** Add 25 µL per well of anti-GFAP– mouse IgG1 antibody and incubate for 3 h at room temperature.
9. **Washing:** Wash three times with PBS.
10. **Fluorescent secondary antibody incubation:** Add 25 µL per well of PhenoVue Fluor goat anti-mouse antibody (5-10 µg/mL) and incubate for 1 h at room temperature, protected from light.

    Optional: At this step, other reagents like PhenoVue Hoechst 33342 Nuclear stain (1-2 µg/mL) or PhenoVue Fluor 555 – Phalloidin (10-50 nM) can be included in the same preparation mix.
11. **Washing:** Wash three times with PBS.
12. **Imaging:** Acquire images using an imaging device of your choice.

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

Assay validation

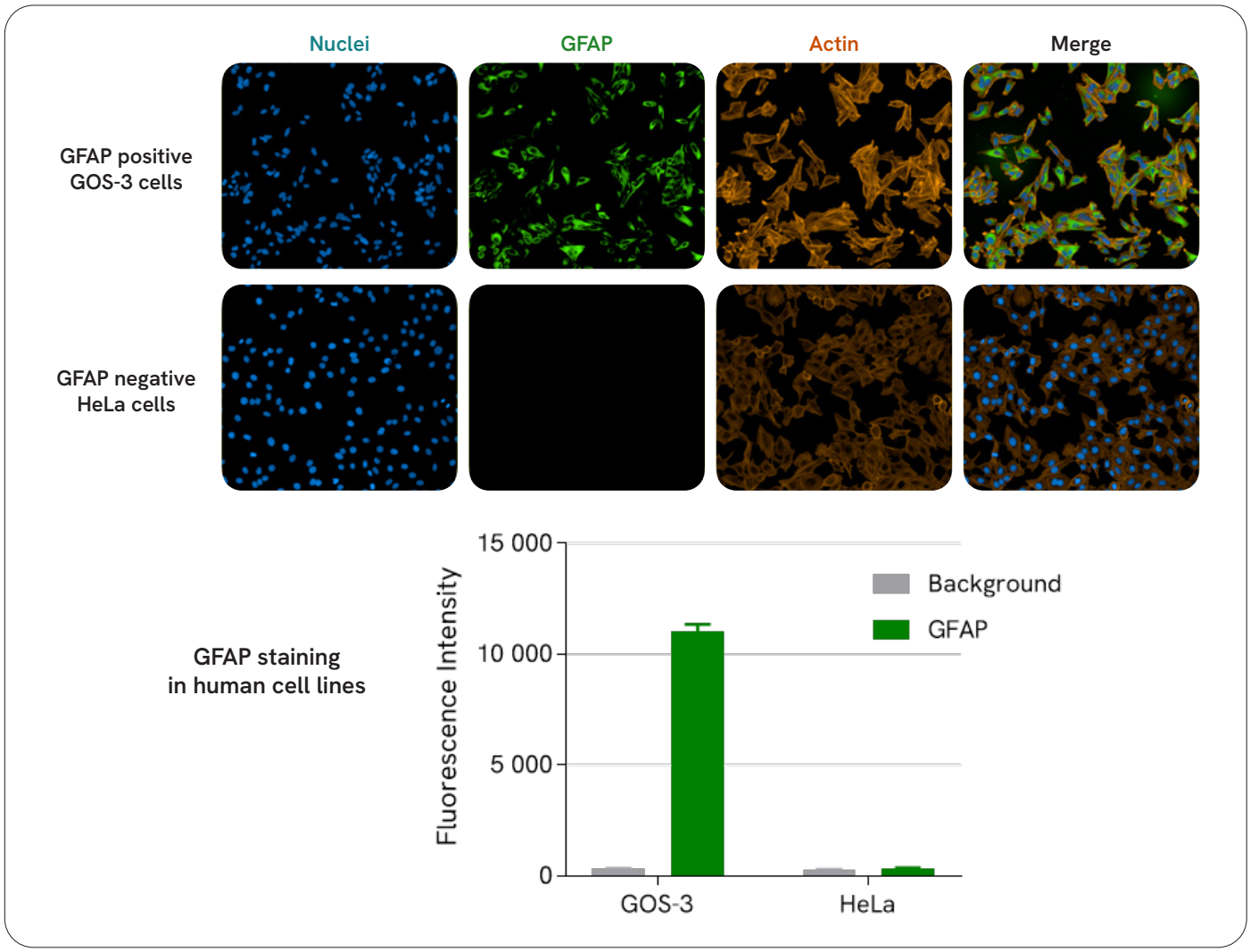


Figure 1: Quantification of GFAP immunostaining in GOS-3 and HeLa cells.

Figure 1 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 Operetta™ CLS 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.

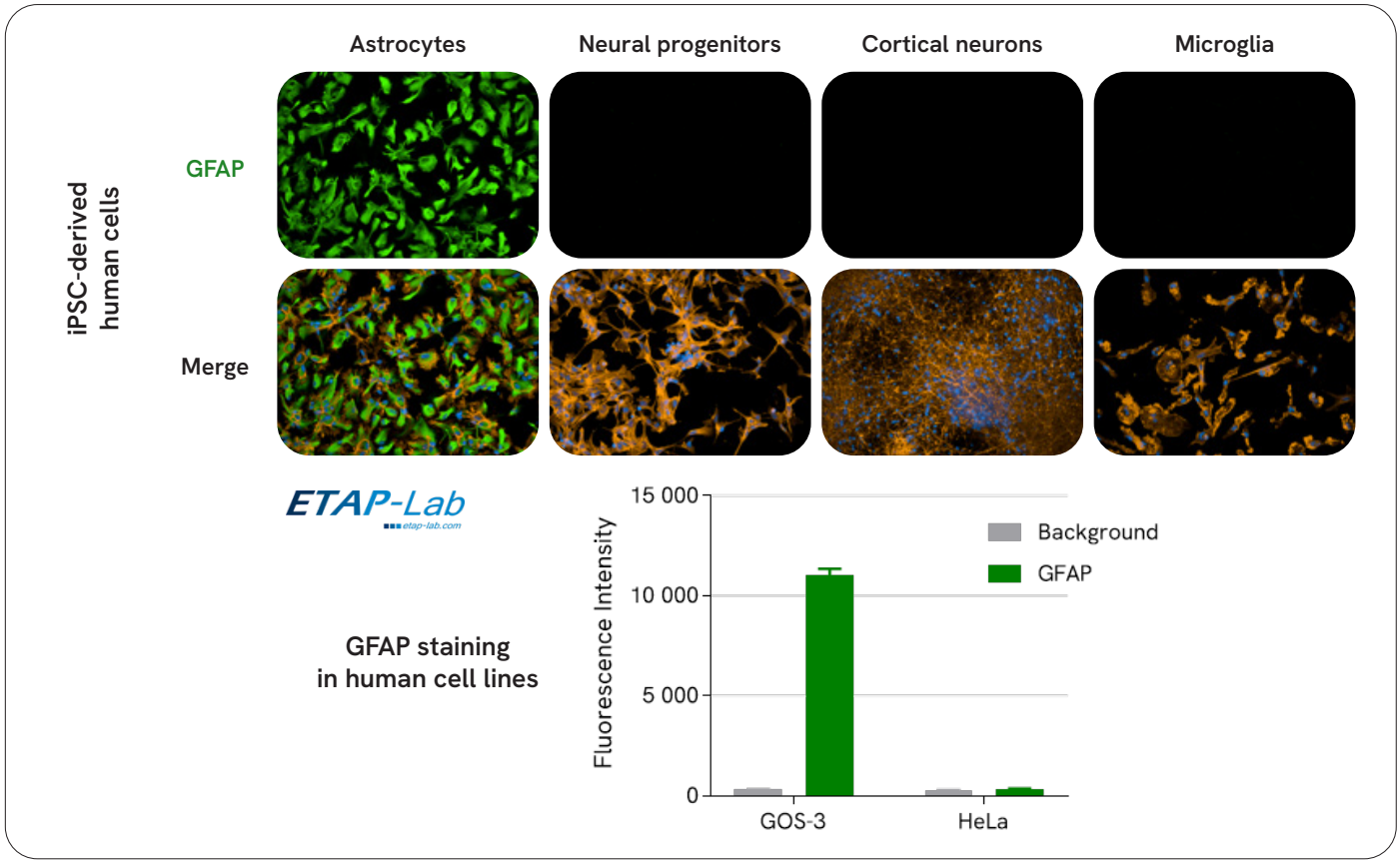


Figure 2: Reliable identification and morphological assessment of various iPSC-derived CNS cell types using the PhenoVue astrocyte differentiation kit.

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. This kit includes antibodies 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.