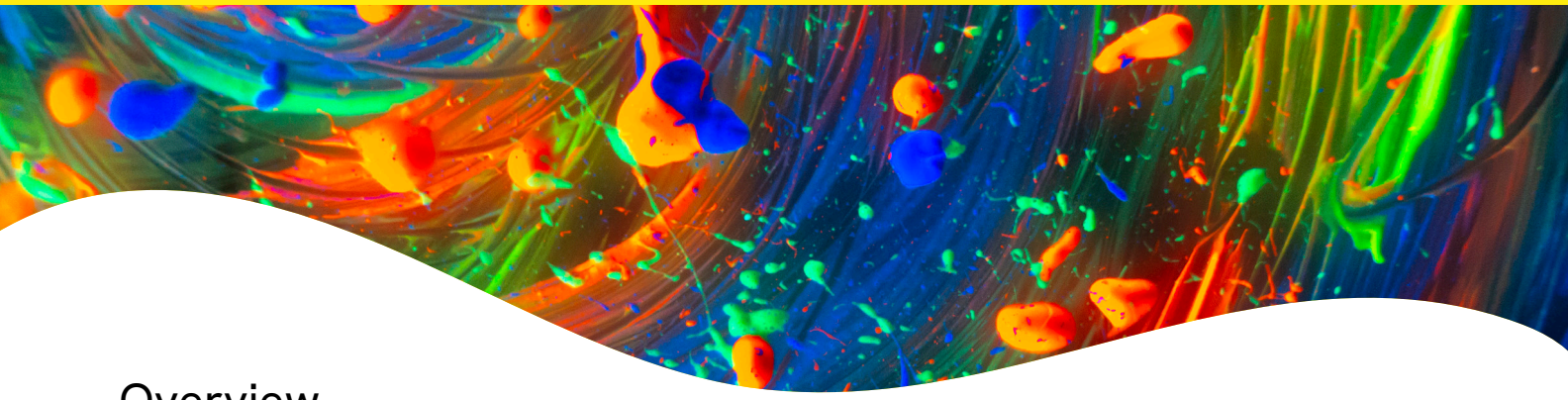
PhenoVue anti-Iba1 antibody



Overview

Iba1 (Ionized calcium-binding adaptor molecule 1), also known as AIF1 (Allograft Inflammatory Factor 1), is a key marker widely used to distinguish microglia from other CNS cells, such as neurons, astrocytes, and oligodendrocytes. This 17 kDa cytoplasmic protein, also expressed in macrophages, binds calcium and actin. While its precise function remains unclear, Iba1 expression is upregulated during microglia activation, nerve injury, CNS ischemia, or in certain brain diseases. Therefore, combining Iba1 expression with morphological characterization can help confirm microglia differentiation or assess their activation status.

The PhenoVue™ anti-Iba1 antibody, included in the PhenoVue Microglia Differentiation Staining Kit (part number: PMIDIF11), can also be used independently in imaging and high-content analysis applications. As an IgG2a antibody, it may serve as an alternative to the PhenoVue anti-β3 Tubulin antibody in the PhenoVue Neuronal Differentiation Staining Kit (part number: PNDIF11). Additionally, it can be used alongside our PhenoVue anti-MAP2 antibody (part number: PABMAP2) and PhenoVue Rat anti-Mouse IgG Isotype Specific Antibody Conjugates (part numbers: 2RTXM488G1H1 and 2RTXM647G2AH1) for microglia/neuron co-culture staining, for example.

Product information

Product name	PhenoVue anti-Iba1 antibody
Part number	PAIBA11
Packaging	1 vial of 100 µL
Concentration	100x
Format	Liquid
Clonality	Monoclonal
Host species	Mouse
Isotype	IgG2a,k
Cross-reactivity	Human
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

1. **Seeding Cells:** Seed cells in PhenoPlate™ 384-well imaging microplates* (or any other suitable cell culture vessel). Incubate under appropriate conditions, typically at 37 °C with 5% CO_2, until the cells reach 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 PhenoVue™ 4% methanol-free paraformaldehyde solution (PVPFA41) directly to the cells and incubate for 10-20 minutes at room temperature.
3. **Washing:** Wash the cells three times with PBS, 5 minutes each wash.
4. **Permeabilization:** Add PhenoVue™ permeabilization solution (PVPERM051), diluted to 0.1% Triton X-100 in PBS, and incubate for 10 minutes at room temperature.
5. **Washing:** Wash three times with PBS, 5 minutes each.
6. **Blocking:** Incubate cells with PhenoVue™ Dye Diluent A (PVDDA1) diluted 1x in PBS for 1 hour at room temperature to block non-specific binding.
7. **Washing:** Wash three times with PBS, 5 minutes each.
8. **Primary Antibody Incubation:** Add 25 µL of mouse monoclonal anti-Iba1 antibody per well and incubate for 1-3 hours at room temperature, or overnight at 4 °C.
9. **Washing:** Wash the cells three times with PBS, 5 minutes each.
10. **Secondary Antibody Incubation:** Add 25 µL per well of PhenoVue™ Fluor Goat anti-Mouse antibody and incubate for 1 hour at room temperature, protected from light.

Optional: At this step, other reagents like PhenoVue™ Hoechst 33342 Nuclear Stain or PhenoVue™ Fluor 555 – Phalloidin can be included in the same preparation mix.

11. **Washing:** Wash three times with PBS, 5 minutes each.
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 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

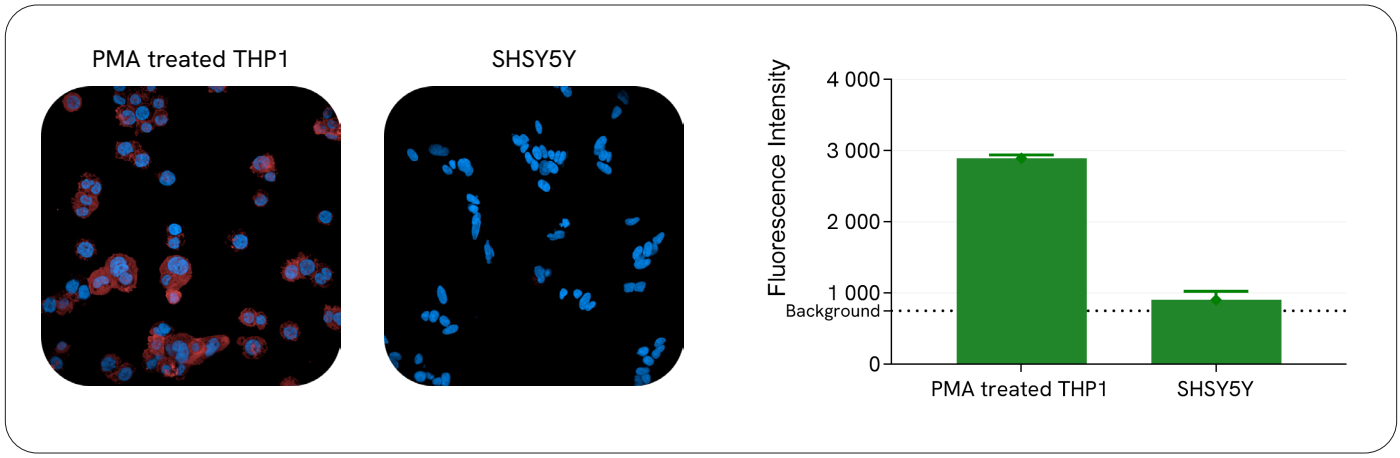


Figure 1: PMA-treated THP1 cells (macrophage-like, positive control) and SHSY5Y cells (neuroblastoma, negative control) were stained with the PhenoVue anti-Iba1 antibody and PhenoVue Fluor 647 Goat anti-Mouse IgG (H+L) highly cross-adsorbed antibody. Images were captured using the Operetta CLS high-content analysis system with an 8-LED light source, a 40x water immersion objective, and confocal mode. As expected, no fluorescent signal was detected in SHSY5Y cells, while the PMA-treated THP1 cells displayed whole-cell labeling, confirming the specificity of the PhenoVue anti-Iba1 antibody.

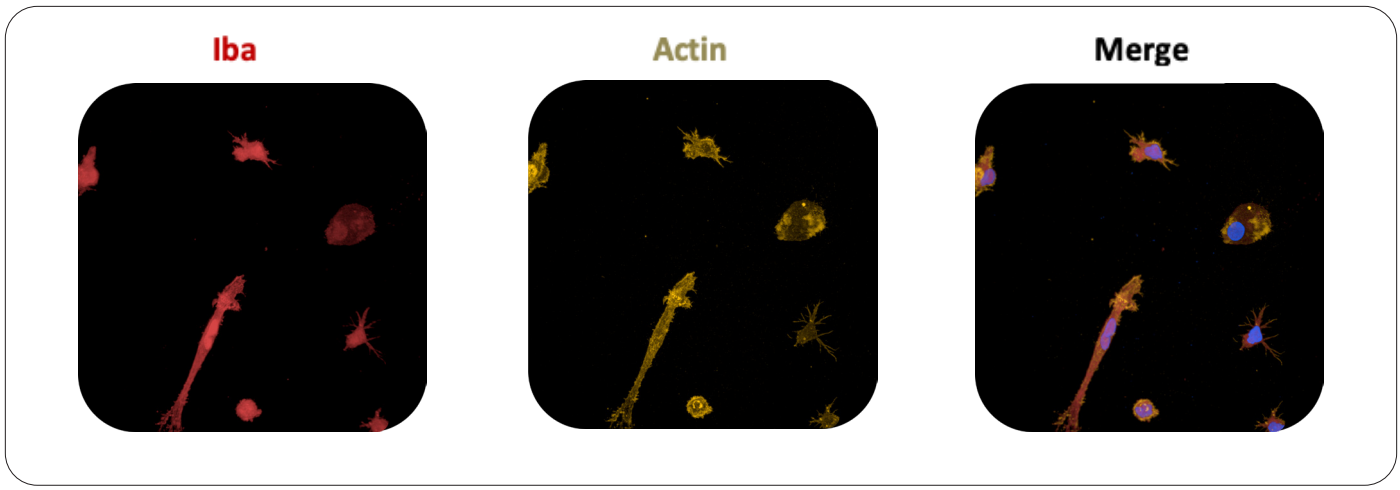


Figure 2: Human iPSC-derived microglia, differentiated and matured for 50 days, were provided by INM collaborators from Montpellier (Coralie Clua Provost and Carole Crozet, INSERM U1298/Université de Montpellier). The cells were seeded in PhenoPlate 96-well microplates at a density of 15,000 cells per well. After fixation, permeabilization, and saturation, the cells were stained using the PhenoVue Microglia Differentiation Staining Kit (part number: PMIDIF11). Images were captured on the Opera Phenix Plus high-content imaging system with 5 lasers, using a 63x water immersion objective in confocal mode.

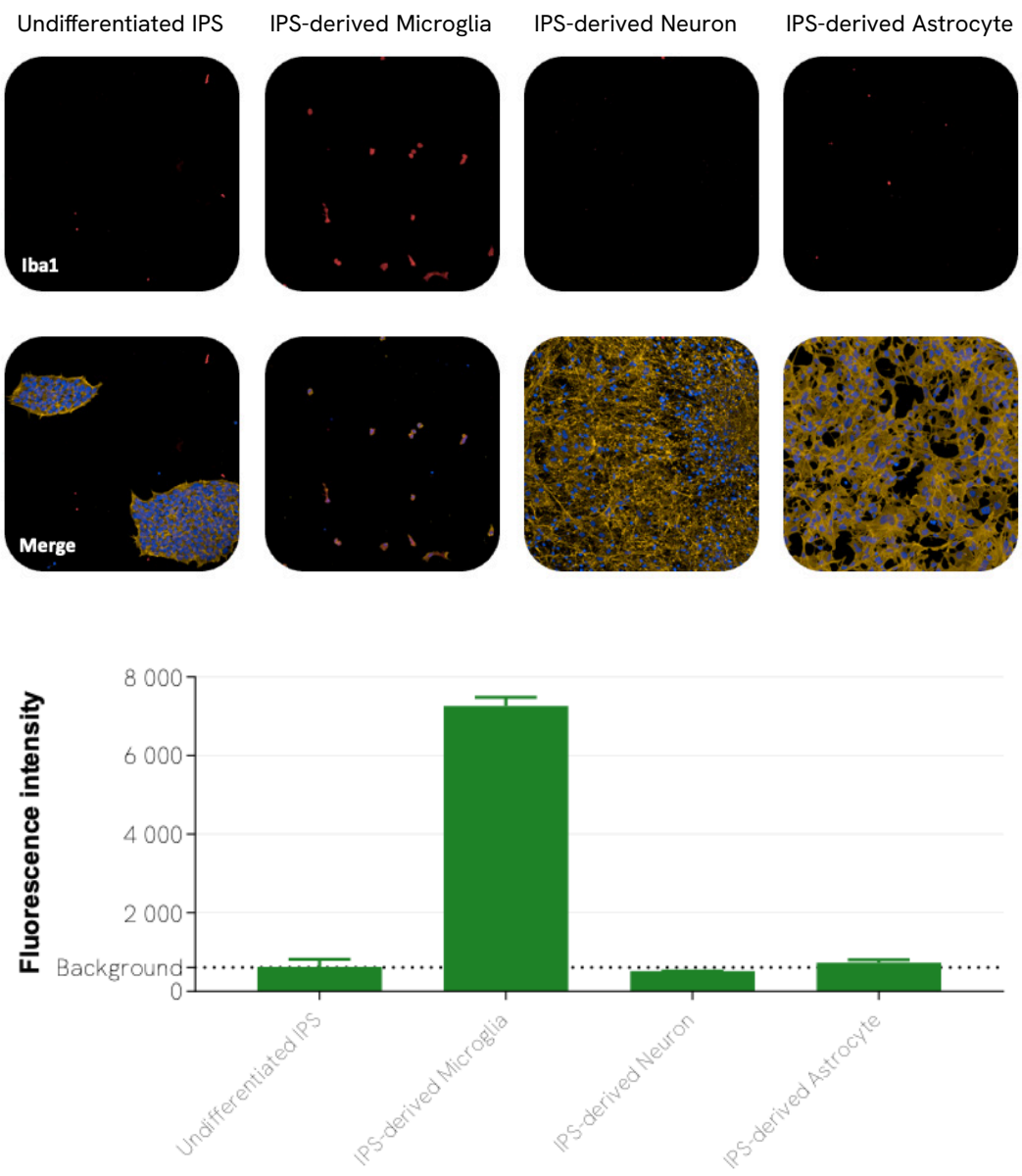


Figure 3: Human undifferentiated IPS (15,000 cells/well), 50 days differentiated and maturated human IPS-derived microglia (15,000 cells/well) and 30 days differentiated human IPS-derived neurons (45,000 cells/well), provided by INM collaborators of Montpellier/Coralie Clua Provost and Carole Crozet INSERM U1298/Université de Montpellier, were seeded in PhenoPlate 96-well microplates, human IPS-derived astrocytes (15,000 cells/well), provided by IXCells, were seeded in PhenoPlate 96-well microplates following their recommended protocol. Cells were then fixed, permeabilized, saturated and stained using the PhenoVue Microglia Differentiation staining kit (part number: PMIDIF11). Images were acquired on the Opera Phenix Plus high-content imaging system (5 lasers) using 20x water objective, confocal mode.