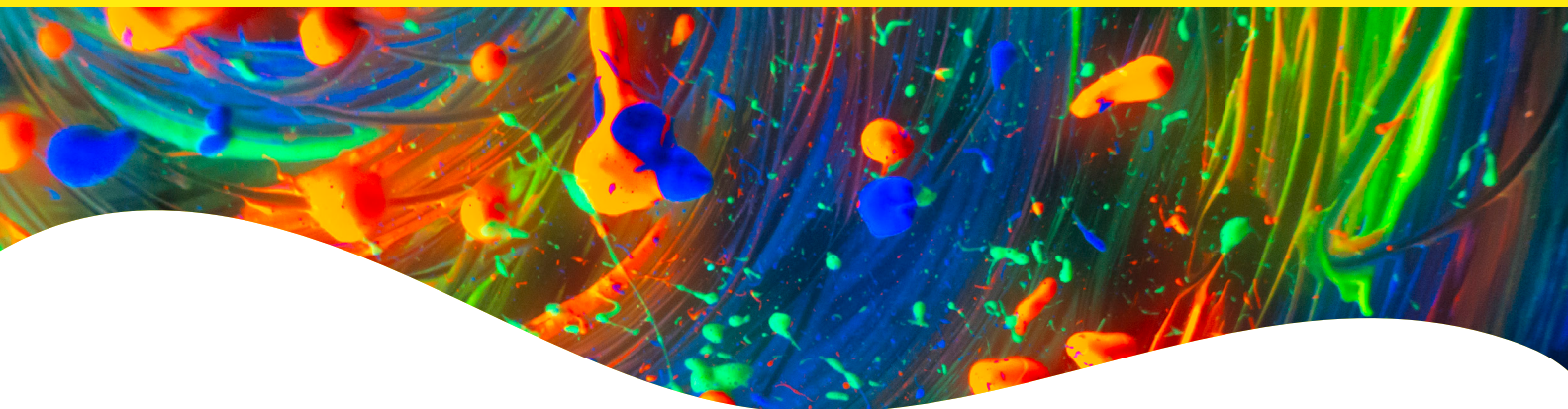




PhenoVue human isotype control antibodies - 2 x 96 wells



Overview

Antibody-mediated internalization is increasingly investigated, particularly in the field of oncology and immuno-oncology. When an antibody binds its target receptor, the antibody-receptor complex is endocytosed into acidic intracellular vesicles. From there, the complex may either recycle back to the cell surface or undergo degradation in lysosomes.

This mechanism is central to the development of antibody-drug conjugates (ADCs), a promising class of anti-cancer drugs. ADCs combine a monoclonal antibody with a cytotoxic payload via a specialized linker. Upon internalization and trafficking to lysosomes, the payload is released, leading to cell death through mechanisms such as DNA damage or microtubule disruption. Efficient and accurate evaluation of antibody internalization is therefore critical.

To confirm that observed effects are specific to antigen binding, appropriate controls are essential. Test antibodies can exhibit off-target interactions, notably via their Fc regions binding to Fc receptors. Isotype control antibodies address this issue by matching the host species and antibody class of the test antibody, while being raised against irrelevant antigens absent from the samples studied. This makes them important for assessing background signal due to non-specific binding.

The PhenoVue™ human IgG1 and IgG4 isotype control antibodies (part numbers PAHIGG1CT1 and PAHIGG4CT1) provide controls for studies using human test antibodies of the same isotype.

Product information

Product name	PhenoVue human IgG1 isotype control antibody - 2x96*	PhenoVue human IgG4 isotype control antibody - 2x96*
Part number	PAHIGG1CT1	PAHIGG4CT1
Packaging	1 vial of 50 µL	1 vial of 50 µL
Concentration	2 mg/mL (13.33µM)**	2 mg/mL (13.33µM)**
Vial format	Liquid	Liquid
Antibody format	Human IgG1, k	Human IgG4, k
Clonality and type	Monoclonal / Recombinant	Monoclonal / Recombinant
Purification	Affinity chromatography	Affinity chromatography
Formulation	PBS pH 7.2	PBS pH 7.2
Applications	High content analysis, immunofluorescence, imaging, microscopy.	High content analysis, immunofluorescence, imaging, microscopy.
Shipping conditions	Room temperature or dry ice	Room temperature or dry ice
Storage conditions	> -16°C	> -16°C

* PhenoVue human isotype control antibodies - 2x96 provide sufficient reagents to stain either 2 x 96-well plates or 1 x 384-well plate at 30 nM concentration.

**Isotype control antibodies are considered at an approximate molecular weight of 150 kDa.

Storage and stability

- Avoid repeated freeze-thaw cycles. After reconstitution, aliquoted reagents must be stored at -16 °C or below.
- 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 12 months.
- The stability of these products is guaranteed until the expiration date provided in the certificate of analysis, when stored as recommended.

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.

Protocol for 96-well imaging plate

PhenoVue human IgG1 and IgG4 isotype control antibodies are compatible with both live-cell and fixed-cell protocols. They are intended to serve as negative controls for test antibodies of the same isotype.

To ensure valid comparison, isotype control antibodies should be processed in parallel with the test antibody, following the same protocol and at the same working concentrations. This allows accurate assessment of background or non-specific binding, ensuring that observed internalization or signal is truly antigen specific.

Assay validation

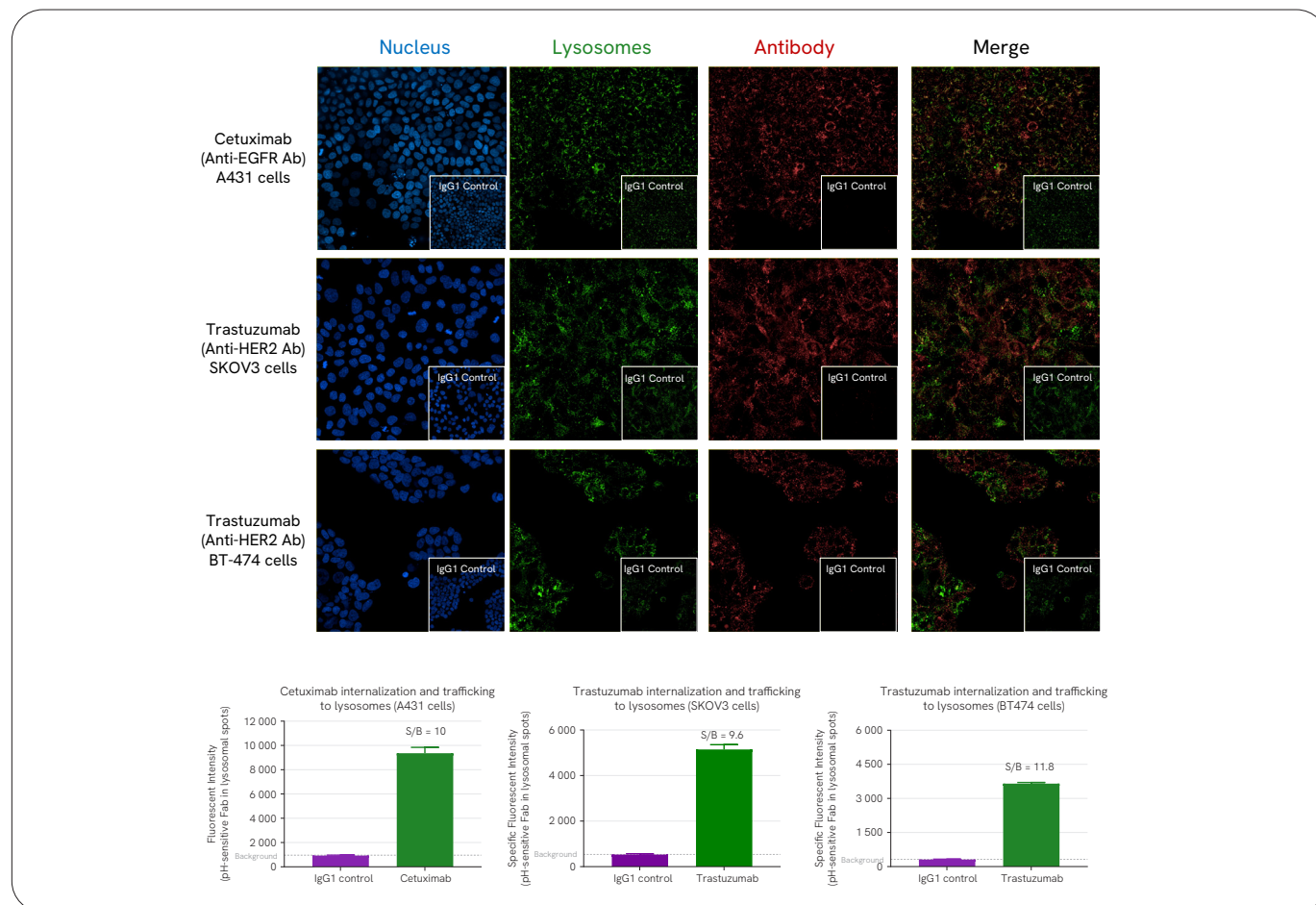


Figure 1. Antibody internalization monitored with the PhenoVue human IgG antibody internalization kit (Fluor 647 pH-sensitive) in different cell models.

Figure 1 shows A431, SKOV3, and BT-474 cells seeded in PhenoPlate 96-well microplates and incubated overnight at 37 °C, 5% CO₂. Cells were then treated in their respective complete culture medium with test human antibodies (anti-EGFR/Cetuximab or anti-HER2/Trastuzumab) or with the PhenoVue human IgG1 isotype control (PAHIGG1CT1), in combination with the PhenoVue human IgG antibody internalization kit (Fluor 647, pH-sensitive) stains. Test antibodies were applied at 4 nM with an antibody:Fab molar ratio of 1:3. In parallel, Fab-only controls (same Fab concentration without test antibody) were included to define the background signal.

Time-lapse imaging was performed over a 24-hour period using the Operetta CLS™ high-content analysis system for A431 cells and the Opera Phenix™ Plus high-content screening system for SKOV3 and BT-474 cells, both with a 40x water-immersion objective in confocal mode. Representative images after 24 h are shown.

Robust internalization of Cetuximab (anti-EGFR) in A431 cells and Trastuzumab (anti-HER2) in SKOV3 and BT-474 cells was observed, with a strong pH-dependent fluorescent signal in lysosomes. In contrast, the IgG1 isotype and Fab-only controls showed minimal background signal. These results demonstrate the kit's ability to specifically quantify antibody uptake and trafficking to acidic lysosomal compartment and discriminate true internalization events from non-specific background.

revvity