



**Fab-pHast human Blue450**  
SECONDARY FLUORESCENT CONJUGATE

*a tool to test antibody specificity, binding, and internalization with results in one (1) day*

**Catalog Number:** PH-01B  
**Quantity:** 100 micrograms, 250 micrograms, 1 milligram  
**Format:** PBS (0.14 M Sodium Chloride; 0.003 M Potassium Chloride; 0.002 M Potassium Phosphate; 0.01 M Sodium Phosphate; pH 7.4), no preservative. Sterile-filtered.  
**Host:** Goat

**Background:** Fab-pHast human Blue is one of our fastest tools for quantitative testing of your primary antibody's specificity, binding, and internalization, providing results in 1 day. Fab-pHast human Blue binds to your primary human antibody via a secondary antibody cross-linked to a pH-dependent fluorescent reporter. This fluorescent reporter will increase intensity as the pH of its surroundings becomes more acidic, as evident when exposed to the environment inside a cell. A successful assay will provide an EC<sub>50</sub> by way of a fluorescence detecting plate reader, illuminating your lead antibody candidates.

**Specificity & Preparation:** This secondary conjugate recognizes YOUR human antibody. Fab-pHast human Blue is a chemical conjugate of goat anti-human monovalent antibody and a pH-dependent fluorescent reporter. The antibodies used to make Fab-pHast human Blue are affinity-purified polyclonal antibodies against both the heavy and light chain of human IgG. The antibody used in this product will cross-react across immunoglobulin classes and subclasses of the same species as they share the same light chain (either kappa or lambda). The pHast fluorescent dye has an excitation wavelength of 362 nm with an emission maxima at 452 nm.

**Usage:** Fab-pHast human Blue generates quantitative testing of the specific, binding, and internalization of your primary human antibody, with results in 1-day. This secondary conjugate is used to evaluate the potential of a primary antibody to internalize.

**There may be lot-to-lot variation in material; working dilutions must be determined by end user. If this is a new lot, you must assess the proper working dilution before beginning a full experimental protocol.**

**Storage:** Gently spin down material 5-10 seconds in a microfuge before use. The material should be stored at 2 -6°C, protected from light. You may add stabilizers such as BSA (1-10 mg/ml) or glycerol for stability and/or preservatives such as sodium azide (2 mM). Under these conditions, the material has a very stable shelf-life. Do not use a reducing agent (such as dithiothreitol, beta-mercaptoethanol or ascorbic acid) with this material. It will inactivate the conjugate.



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all product  
references.

#### Selected References:

1. Kinneer K, Meekin J, Tiberghien AC, Tai YT, Howard PW, Phipps S, Kiefer CM, Rebelatto MC, Dimasi N, Moriarty A, Papadopoulos KP, Sridhar S, Gregson SJ, Wick MJ, Masterson L, Anderson KC, Herbst R, Tice DA. (2018) SLC46A3 as a Potential Predictive Biomarker for Antibody-Drug Conjugates Bearing Noncleavable Linked Maytansinoid and Pyrrolobenzodiazepine Warheads. *Clin Cancer Res* 24(24):6570-6582.

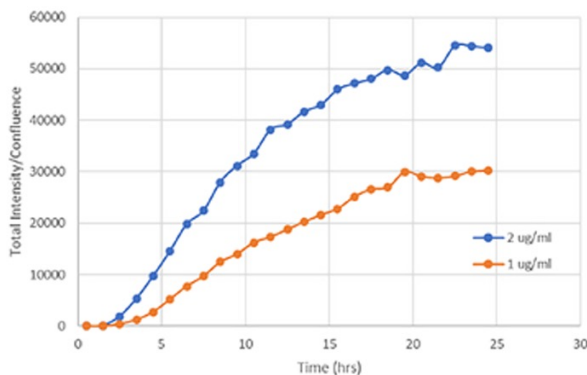
**Control(s):** The recommended control for use with this product would be a non binding primary antibody, such as an isotype control, that mimics your primary antibody targeting agent. This control antibody should be used with Fab-pHast identically to the manner in which you test your primary antibody of interest.

#### Safety:

Good laboratory technique must be employed for safe handling of this product. This requires observation of the following practices:

1. Wear appropriate laboratory attire, including lab coat, gloves and safety glasses.
2. Do not pipet by mouth, inhale, ingest or allow product to come into contact with open wounds. Wash thoroughly any part of the body which comes into contact with the product.
3. Avoid accidental autoinjection by exercising extreme care when handling in conjunction with any injection device.
4. This product is intended for research use by qualified personnel only. It is not intended for use in humans or as a diagnostic agent. Advanced Targeting Systems is not liable for any damages resulting from the misuse or handling of this product.

To view protocol(s) for this and other products please visit: [www.ATSBio.com/library/protocols](http://www.ATSBio.com/library/protocols)



Fab-pHast fluorescence was monitored over time using an IncuCyte SX5. Fluorescence intensity increases as Fab-pHast becomes activated in the acidic environment of the endocytic pathway, indicating internalization and trafficking. Data generated using an IncuCyte SX5.