



**FabFc-pHast human**  
SECONDARY FLUORESCENT CONJUGATE

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

**Catalog Number:** PH-10  
**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:** FabFc-pHast human is one of our fastest tools for quantitative testing of your primary antibody's specificity, binding, and internalization, providing results in 1 day. FabFc-pHast human 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. The FabFc-pHast products can be used in B-cell research with the confidence that fluorescence is primary antibody-mediated.

**Specificity & Preparation:** This secondary conjugate recognizes YOUR human antibody. FabFc-pHast is a chemical conjugate of goat anti-human monovalent antibody and a pH-dependent fluorescent reporter. The antibodies used to make FabFc-pHast human are affinity-purified polyclonal antibodies against Fc (gamma) portion of the human IgG. The antibody used in this product will not cross-react with the Fab portion of human IgG, or human IgM or IgA, but may cross-react with Ig from other species. The pHast fluorescent dye has an excitation wavelength of 532 nm with an emission maxima at 560 nm.

**Usage:** FabFc-pHast human 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.



## FabFc-pHast human SECONDARY FLUORESCENT 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.

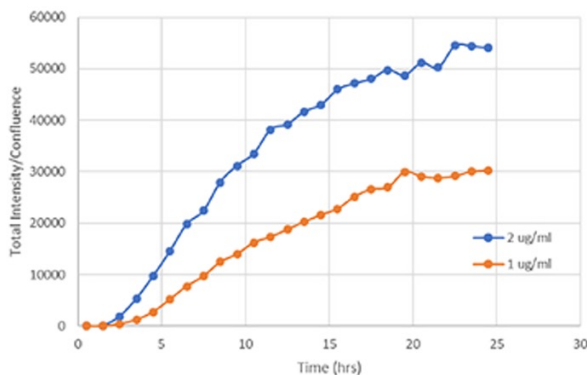
**Control(s):** The recommended control for use with this product would be a non binding primary antibody, such as an isotype control, that mimics your primary antibody targeting agent. This control antibody should be used with FabFc-pHast identically to the manner in which you test your primary antibody 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 human (Cat. #PH-01) 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. Fab-pHast reacts with whole molecule IgG (heavy and light chains). 1.5 ml "Fab-pHast spiked solution" will use about 43 micrograms of Fab pHast. This is assuming a 500-nM spiked solution and performing an assay on a 96-well plate, which is the suggested amount on the protocol. This is enough to test 1 antibody in sextuplicate (6 data points) at 8 different concentrations of antibody. Data generated using an InCuCyte SX5. The data were as expected with a good signal to noise ratio.