Advanced Targeting Systems

6352 CORTE DEL ABETO, STE B
CARLSBAD, CA 92011 USA
01.858.642.1988 • WWW.ATSBIO.COM

Fab-pHast mouse
SECONDARY FLUORESCENT CONJUGATE
a tool to test antibody specificity, binding, and internalization with results in one (1) day

Catalog Number: PH-02
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 mouse 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 mouse binds to your primary mouse 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 EC50 by way of a fluorescence detecting plate reader, illuminating your lead antibody candidates.

Specificity & Preparation: This secondary conjugate recognizes YOUR mouse antibody. Fab-pHast is a chemical conjugate of goat anti-mouse monovalent antibody and a pH-dependent fluorescent reporter. The antibodies used to make Fab-pHast mouse are affinity-purified polyclonal antibodies against both the heavy and light chain of mouse 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 532 nm with an emission maxima at 560 nm.

Usage: Fab-pHast mouse generates quantitative testing of the specific, binding, and internalization of your primary mouse 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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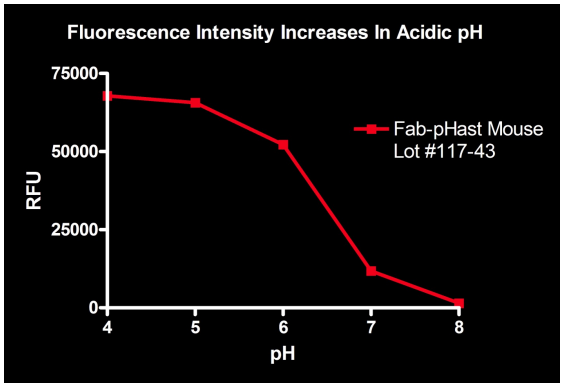
Scan to view all product references.

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.

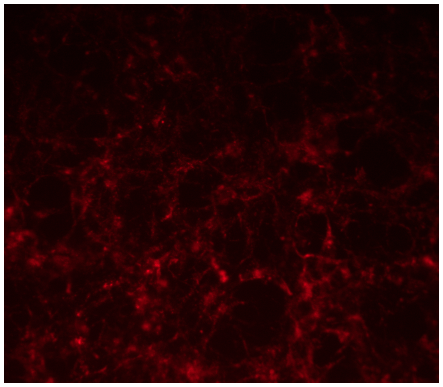
Safety:
Good laboratory technique must be employed for safe handling of this product. This requires observation of the following practices:

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



pH response of Fab-pHast Mouse: Fluorescence (RFU) is shown as a function of pH for Fab-pHast Mouse (Cat. PH-02). The more acidic pH shows a large amount of fluorescence, while the basic pH shows almost no fluorescence. 1 μl, or 0.9 μg, was added to 99 μl of 50 mM potassium phosphate at various pH's in a bottom read 96-well plate. Plates were mixed gently on a plate mixer for 5 minutes at room temperature and read on a Spectra Max Gemini EM (Ex: 532nm/Em: 560nm).



Fluorescent imagery of Fab-pHast Mouse conjugated to 192-IgG: HEK-293 cells, transfected with the p75 neurotrophin receptor, were plated at 20,000 cells/well in a 96-well plate and allowed to adhere overnight. 10 nM of the 192-IgG antibody (Cat. #AB-N43) was incubated at room temperature with 30 nM of Fab-pHast Mouse (Cat. #PH-02) for 20 minutes. The Fab-pHast conjugated antibody was then added to the cells and gently mixed for 2 min on a plate mixer. Cells were incubated overnight to allow internalization, although internalization could be detected within a few hours. Media was replaced with PBS to achieve a higher sensitivity and then the cells were analyzed on a fluorescent microscope under 20X magnification using a Y3 Leica filter cube.