



Streptavidin-pHast
SECONDARY FLUORESCENT CONJUGATE

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

Catalog Number: PH-03
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.

Background: Streptavidin is a tetrameric protein (molecular weight 53 kDa in its recombinant form), with each subunit able to bind a single biotin molecule. The bond between streptavidin and biotin is rapid and essentially non-reversible, unaffected by most extremes of pH, organic solvents, and denaturing reagents. It is the strongest known noncovalent biological interaction ($K_a = 10^{15} \text{ M}^{-1}$) between protein and ligand. The streptavidin used to make Streptavidin-pHast contains no carbohydrate group and has a neutral isoelectric point, which therefore reduces the nonspecific binding as compared to avidin. A variety of molecules, including lectins, proteins, and antibodies, can be biotinylated and reacted with streptavidin-labeled probes or other detection reagents for use in biological assays.

Streptavidin-pHast is one of our fastest tools for quantitative testing of your biotinylated protein's specificity, binding, and internalization, providing results in 1 day. Streptavidin-pHast binds to your biotinylated protein and contains a pH-dependent fluorescent reporter (pHast). 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.

Specificity & Preparation: This secondary conjugate recognizes YOUR biotinylated protein. Streptavidin-pHast is a conjugate of streptavidin and a pH-dependent fluorescent reporter. The pHast fluorescent dye has an excitation wavelength of 532 nm with an emission maxima at 560 nm.

Usage: Streptavidin-pHast generates results within 1-day by testing specific binding and internalization of your biotinylated protein. This secondary conjugate is used to evaluate the potential of your biotinylated protein 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.

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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references.

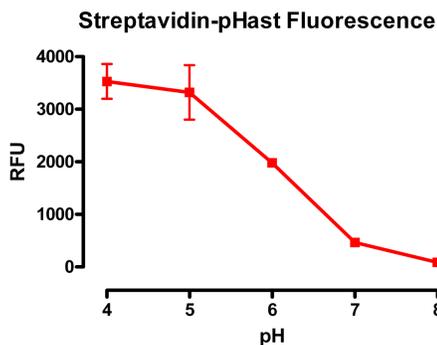
Control(s): The recommended control for use with this product would be a non binding biotinylated protein, such as an isotype control, that mimics your biotinylated protein targeting agent. This control should be used with Streptavidin-pHast identically to the manner in which you test your biotinylated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



Fluorescence (RFU) is shown as a function of pH for Streptavidin-pHast

Streptavidin-pHast was diluted 1:100 in 50 mM potassium phosphate with varying pH levels. The more acidic pH shows a large amount of fluorescence, while the basic pH shows almost no fluorescence. Streptavidin-pHast will fluoresce in the endosomes and lysosomes of cells due to their more acidic pH. Plates read on a Spectra Max Gemini EM (Ex: 532nm/EM: 560nm).