ADVANCED TARGETING SYSTEMS

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Streptavidin-ZAP

ZAP CONJUGATE

a tool to "piggyback" onto YOUR biotinylated material via streptavidin;
targeting cells that recognize YOUR biotinylated material, eliminated via saporin

Catalog Number: IT-27
Quantity: 25 micrograms, 100 micrograms, 250 micrograms
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 ($Ka = 10^{15}\ M^{-1}$) between protein and ligand. The streptavidin used to make Streptavidin-ZAP 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-ZAP "piggybacks" onto YOUR biotinylated material in order to evaluate the ability of the reagent to internalize upon binding to its receptor. Once the conjugate is internalized, saporin breaks away from the targeting agent and inactivates the ribosomes, which causes protein inhibition and, ultimately, cell death. Potency may vary according to the specificity and affinity or YOUR material to ITS receptor. When your *in vitro* results confirm the desired specificity, it is recommended that you order a custom conjugation of your material directly to saporin.

Specificity and Preparation:
This conjugate recognizes cells targeted by biotinylated materials. Streptavidin-ZAP is a chemical conjugate of streptavidin and the ribosome-inactivating protein, saporin. This product is routinely tested by cytotoxicity assay.

Usage and Storage:
Streptavidin-ZAP converts biotinylated materials into targeted toxins. **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.** You may premix undiluted streptavidin-ZAP with your undiluted biotinylated material at a 1:1 molar ratio and store this mixture at -20°C until ready for use. We recommend only diluting the material right before use.
Gently spin down material before use; 5-10 seconds in a microfuge should be adequate. The material should be stored at -20°C in undiluted aliquots. Material should be aliquoted to a convenient volume and quantity to avoid repeated freezing and thawing that can damage the protein content. Under these conditions, the material has a very stable shelf-life. Thawing should be done at room temperature or on ice. The thawed solution should remain on ice until use.
Do not use a reducing agent (such as dithiothreitol, beta-mercaptoethanol or ascorbic acid) with this material. It will inactivate the toxin.
If the biotinylated targeting agent is recognized by a human receptor, this material will be toxic to human cells expressing the appropriate receptor. Handling should be done by experienced personnel. Gloves and safety glasses are required when handling this product. Care in disposal is mandatory; autoclaving or exposure to 0.2 M sodium hydroxide will inactivate the material. All labware that comes into contact with this material should be likewise treated.

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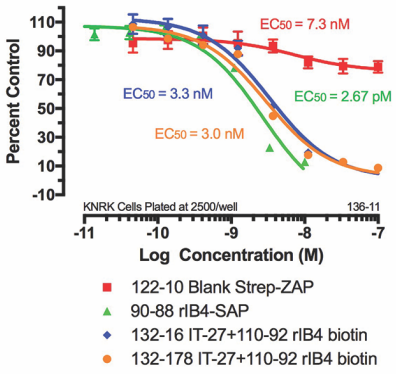
Available Control(s): Saporin

References:
1. Sheriff ST, Xiao C, Chance WT, Kasckow JW, Balasubramaniam A (2002) Selective lesion of neuropeptide Y (NPY)-receptor neurons in hypothalamus inhibit food intake and reduces body weight in rats. *Soc Neurosci Mtg, Orlando FL*, Abstract #384.1.
2. Kohls M (2006) Evaluate Potential Targeting Molecules. *Nature Methods*. http://www.nature.com/app_notes/nmeth/2006/063006/full/an1788.html.

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/support/protocols



KNRK cells are plated at 2500 cells/well and incubated overnight. Streptavidin-ZAP is premixed with Biotinylated-IB4 in equimolar concentrations. Blank-Streptavidin-SAP, IB4-SAP, and the Streptavidin-ZAP + Biotinylated-IB4 mixture are then added in 10 ul volumes and the plates incubated 72 hours. XTT/PMS mixture added at 50 ul volumes to each well and plates read at 450 nm and data analysis done with Prism software (GraphPad, San Diego).