Advanced Targeting Systems

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MonoBiotin-ZAP

ZAP CONJUGATE

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

Catalog Number: BT-ZAP
Quantity: 50 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: Saporin is obtained from the seeds of the Soapwort plant (*Saponaria officinalis*), a plant that grows wildly in Britain and other parts of Europe. Saporin is a plant enzyme with N-glycosidase activity that depurinates a specific nucleotide in the ribosomal RNA 28S, thus irreversibly blocking protein synthesis. It belongs to the well-characterized family of ribosome-inactivating proteins (RIPs). There are two types of RIPs: type I, which are much less cytotoxic due to the lack of the B chain and type II, which are distinguished from type I RIPs by the presence of the B chain and their ability to enter cells on their own. However, type I RIPs can still be internalized by fluid-phase endocytosis. Upon internalization, the ribosomes are inactivated, resulting in cell death. 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.
BT-ZAP "piggybacks" onto YOUR streptavidin conjugates 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 & Preparation: This conjugate recognizes streptavidin conjugates. MonoBiotin-ZAP (BT-ZAP) is a chemical conjugate of saporin labeled with biotin in a 1:1 chemically-determined average molar ratio.

Usage: MonoBiotin-ZAP uses YOUR streptavidinylated targeting agent to target and eliminate cells. This secondary conjugate is used to evaluate the potential of a streptavidinylated targeting agent 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. Aliquot and store frozen at -20 or -80°C. Avoid repeated freezing and thawing. For disposal: autoclave, or expose to 0.2 M NaOH, materials that come into contact with the toxin.
Do not use a reducing agent (such as dithiothreitol, beta-mercaptoethanol or ascorbic acid) with this material. It will inactivate the toxin.
If the streptavidinylated 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.

For research use only, not for use as a diagnostic.

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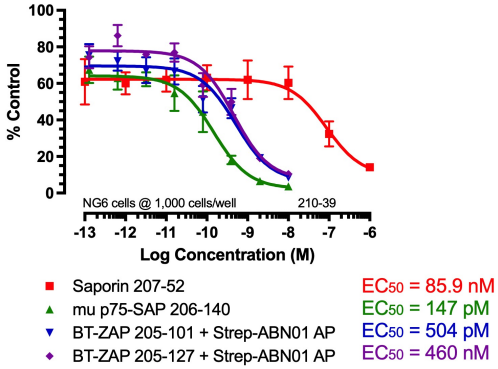
Selected References:

1. Minami SS, Sun B, Popat K, Kauppinen T, Pleiss M, Zhou Y, Ward ME, Floreancig P, Mucke L, Desai T, Gan L. (2012) Selective targeting of microglia by quantum dots. *J Neuroinflammation* 9:22.
2. Stirpe F, Barbieri L, Battelli MG, Soria M, Lappi DA. (1992) Ribosome-inactivating proteins from plants: present status and future prospects. *Bio/Technol* 10:405-412.
3. Lappi DA, Esch FS, Barbieri L, Stirpe F, Soria M. (1985) Characterization of a *Saponaria officinalis* seed ribosome-inactivating protein: immunoreactivity and sequence homologies. *Biochem Biophys Res Commun* 129:934-942.
4. Stirpe F, Gasperi-Campani A, Barbieri L, Falasca A, Abbondanza A, Stevens WA. (1983) Ribosome-inactivating proteins from the seeds of *Saponaria officinalis* L. (soapwort) of *Agrostemma githago* L. (corn cockle) and of *Asparagus officinalis* (asparagus) and from the latex of *Hura crepitans* L. (sandbox tree). *Biochem J* 216:617-625.

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



NG6 cells were plated at 1000 cells/90 µl/well and incubated overnight. Saporin and mu p75-SAP (Cat. #IT-16) dilutions were made in cell media and 10 µl was added to each well. Mono-Biotin-ZAP (Cat. #BT-ZAP) was reacted equimolar with streptavidin-anti-mu p75 and then added to plates. The plates were incubated for 72 hours. The plates were developed using a solution of XTT/PMS and read at 450 nm. Cytotoxicity was analyzed by comparing well readings of the treated wells to those of the control wells, expressed as a percentage. The number of viable cells remaining on the day of development is measured via cell metabolism of a colorimetric molecule within the developing reagents. Analysis was performed using Prism software (GraphPad, San Diego).

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