ADVANCED TARGETING SYSTEMS

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Anti-6 His-ZAP

TAG-TARGETED TOXIN

a tool for eliminating 6 His-expressing cells or to "piggyback" onto your 6-His tagged proteins; targeted via mouse monoclonal antibody to 6 His, eliminated via saporin

Catalog Number: IT-52
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:
Tag-targeted toxins are powerful and specific lesioning agents used in the technique known as Molecular Surgery. The ribosome-inactivating protein, saporin (from the seeds of the plant, *Saponaria officinalis*) is bound to a targeting agent that "piggybacks" onto YOUR cells expressing proteins tagged with 6 His. Once the conjugate is internalized, saporin breaks away from the targeting agent, and inactivates the ribosomes which causes protein inhibition and, ultimately, cell death. Cells that do not have the cell surface marker are not affected.
The use of polyhistidine tags has become a popular method for protein purification, commonly used in the screening process as a tag for your protein or peptide of interest. This polyhistidine epitope tag is generally comprised of six consecutive histidine amino acid residues located at the N-terminal, C-terminal, or internally. The 6-His-Tag is widely used because of its affinity to bind nickel or cobalt metal ions attached to sepharose, which can then be used to purify the protein in a native or denatured state.

Specificity and Preparation:
This tag-targeted toxin recognizes YOUR 6 His-tagged recombinant proteins or 6 His-tagged proteins over-expressed in cells. Anti-6-His-ZAP is a chemical conjugate of the mouse monoclonal antibody to 6 His and the ribosome-inactivating protein, saporin.

Usage and Storage:
Anti-6 His-ZAP specifically eliminates YOUR 6 His-tagged recombinant proteins or 6 His-tagged proteins over-expressed in cells. **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.**
Gently spin down material before use; 5-10 seconds in a microfuge should be adequate. Store the material in undiluted aliquots at –20°C for 1-2 months. For longer term storage store the material at –80°C. 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.
This material is an extremely potent cytotoxin. 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.
Note: When used in a cytotoxicity assay, un-bound primary antibody will compete with primary antibody bound to Anti-6 His-ZAP and may reduce cytotoxicity through competitive inhibition of the primary antibody-secondary conjugate complex.

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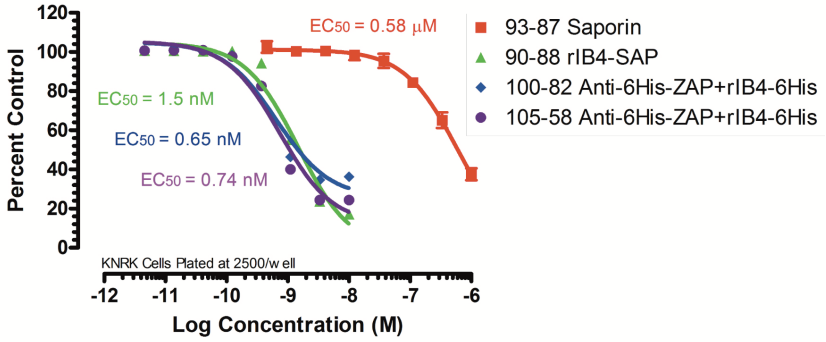
Available Control(s): Mouse IgG-SAP

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 were plated at 2500 cells/90 µl/well and incubated overnight at 37°C. Next day, Saporin and rIB4-SAP dilutions were made in cell media, and 10 µl was added to each well. rIB4-6 His was diluted in cell media containing, at a final concentration, 100 ng/10 µl Anti-6 His-ZAP, and 10 µl was added to each well. The plates incubated 72 hours at 37°C. To develop, the medium was removed from the plate, and the cells were fixed with 10% TCA for 1 hour at 4°C. The plates were washed 3 times with tap water and allowed to air dry. 50 µl of 0.4% sulfarhodamine B/1% acetic acid was added to each well and the plate incubated for 30 min at room temperature. The plates were washed 3 times with 1% acetic acid and allowed to air dry. The dye was solubilized with 100 µl of 10 mM unbuffered tris base per well, with 5 min of gentle shaking. The plates were read at 564 nm, and data analysis was done with Prism software (GraphPad, San Diego)