ADVANCED TARGETING SYSTEMS

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# IB4-SAP

TARGETED SAP CONJUGATE

*a tool for eliminating cells that express α-D-galactopyranoside residues in cells; targeted via recombinant Isolectin B4 (IB4), eliminated via saporin*

**Catalog Number:** IT-10
**Quantity:** 25 micrograms, 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:**
Targeted SAP conjugates 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 (anything that is recognized on the cell surface and internalized). The targeted conjugate is administered to cells (*in vitro* or *in vivo*). The targeting agent seeks out and binds to its target on the cell surface. 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.
Isolectin B4 (IB4) is one of a family of five alpha-D-galactose-binding lectins from *Griffonia (Bandeiraea) simplicifolia*. Recombinant IB4* was expressed in *E. coli* and purified using affinity chromatography. In one important application IB4-SAP specifically eliminates the IB4-positive c-fiber nociceptor neurons, while sparing the peptidergic neurons. Upon binding the alpha-D-galactopyranoside residues expressed on the cell surface, IB4-SAP becomes internalized and saporin inhibits protein synthesis, resulting in elimination of the neurons. The cytotoxin is extremely potent, with an $ED_{50}$ in the low picomolar range for some alpha-D-galactopyranoside-expressing cells *in vitro*. IB4-SAP is an excellent tool for the study of pain transmission and the biological roles of IB4+ cells *in vivo*.
* patent pending

**Specificity and Preparation:**
This targeted toxin recognizes α-D-galactopyranoside-positive cells. IB4-SAP is a chemical conjugate of recombinant IB4 expressed in *E. coli* and the ribosome-inactivating protein, saporin (Cat. #PR-01). This product is routinely tested by cytotoxicity assay.

**Usage and Storage:**
IB4-SAP eliminates α-D-galactopyranoside-positive cells. All other cells are left untouched. **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.

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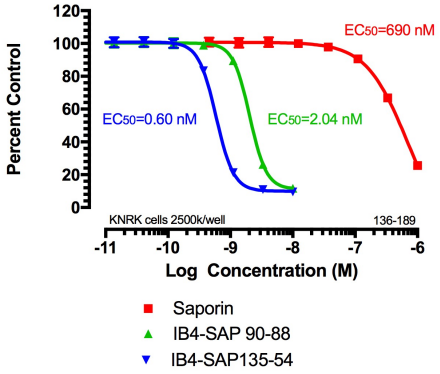
**Available Control(s):** Saporin

**References:**
1. Ye Y, Bae S, Viet CT, Troob S, Bernabe D, Schmidt BL. (2014) IB4(+) and TRPV1(+) sensory neurons mediate pain but not proliferation in a mouse model of squamous cell carcinoma. *Behav Brain Funct* 10 (1):5.
2. Vulchanova L, Olson TH, Stone LS, Riedl MS, Elde R, Honda CN (2001) Cytotoxic targeting of isolectin IB4-binding sensory neurons. *Neurosci* 108(1):143-155.
3. Tarpley JW, Martin WJ, Baldwin BS, Forrest MJ, MacIntyre DE (2000) Contribution of IB-4-positive sensory neurons to NGF-induced hyperalgesia in the rat. *Soc Neurosci Mtg, New Orleans LA,* Abstract #633.18.
4. Basbaum AI (1999) Distinct neurochemical features of acute and persistent pain. *Proc Natl Acad Sci USA* 96:7739-7743.
5. Snider WD and McMahon SB (1998) Tackling pain at the source: New ideas about nociceptors. *Neuron* 20:629-632.

**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/well in 96-well plates and allowed to acclimate overnight at 37C. Saporin alone (Cat. #PR-01) was used as a negative control. The highest concentration of Saporin used in the assay was 1 uM and serially diluted 1:3 across the plate. Concentrations of IB4-SAP started at 10 nM and were serially diluted 1:3 across the plate. Dilutions were tested in replicates of six with error bars representing the standard deviation (SD). Treatment of the cells was for 72 hours. Assay was developed using XTT/PMS and were read at 450 nm. Data analysis done with Prism (GraphPad, San Diego).