Advanced Targeting Systems

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CTB-SAP

TARGETED SAP CONJUGATE

a tool for eliminating cells that express the GM1 receptor;
targeted via cholera toxin B-subunit, eliminated via saporin

Catalog Number: IT-14
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.
The GM1 (Galactosyl-N-Acetylgalactosaminyl) receptor is found on cells such as motoneurons, sympathetic pre-ganglionic neurons, astrocytes as well as cells in the intestinal epithelium. Cholera toxin B (CTB) binds the GM1 receptor and administration of CTB-SAP causes ablation of the specific cells expressing the receptor. CTB-SAP can be used to create a model of respiratory motor neuron death to establish an animal model of Amyotrophic lateral sclerosis (ALS), otherwise known as Lou Gehrig's disease. This model also demonstrates that CTB-SAP is retrogradely transported. Intrathecal injection of CTB-SAP results in elimination of oligodendrocytes and astrocytes as well as supporting the study of demyelinating conditions.

Specificity & Preparation: This targeted toxin recognizes GM_1 ganglioside (cell membrane component). CTB-SAP is a chemical conjugate of cholera toxin B-subunit and the ribosome-inactivating protein, saporin. This product is routinely tested using a cytotoxicity assay on HS294T cells.

Usage: Applications include ablation of any cell type expressing the GM_1 receptor, including preganglionic neurons,[1] motoneurons,[2,3] and astrocytes.[4] **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. 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.

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

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Scan to view all product references.

Selected References:

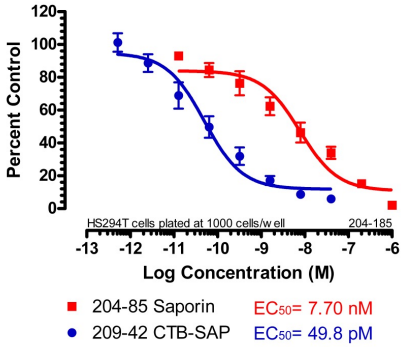
1. Wu M, Kc P, Mack SO, Haxhiu MA (2005) Ablation of vagal preganglionic neurons innervating the extra-thoracic trachea affects ventilatory responses to hypercapnia and hypoxia. *Respir Physiol Neurobiol* [Aug 10 Epub].
2. Fargo KN, Sengelaub DR (2004) Testosterone manipulation protects motoneurons from dendritic atrophy after contralateral motoneuron depletion. *J Comp Neurol* 469(1):96-106.
3. Fargo KN, Sengelaub DR (2004) Exogenous testosterone prevents motoneuron atrophy induced by contralateral motoneuron depletion. *J Neurobiol* 60(3):348-359.
4. Jasmin L, Janni G, Moallem TM, Lappi DA, Ohara PT (2000) Schwann cells are removed from the spinal cord after effecting recovery from paraplegia. *J Neurosci* 20(24):9215-9223.
5. Llewellyn-Smith IJ, Martin CL, Arnolda LF, Minson JB (1999) Retrogradely transported CTB-saporin kills sympathetic preganglionic neurons. *Neuroreport* 10(2):307-312. doi: 10.1097/00001756-199902050-00019 PMID: 10203327

Control(s): Saporin

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



HS294T cells were plated at 1000 cells per 90 ul/well in a 96-well plate and incubated overnight. Saporin (Cat. #PR-01) starting concentration was 1 micromolar and serially diluted 1:5. CTB-SAP starting concentration was 10 nanomolar and serially diluted 1:5. Saporin and CTB-SAP were added in 10 ul volumes and the plates left to incubate for 72 hours. XTT/PMS developing reagent was added and the plates read at 450 nm. Data analyzed by Prizm (GraphPad).

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U.S. Patent 6.376,460 "Method of Modulating Cellular Activity" protects methods of peripheral injection of subjects using this neuronal tracer. Before using these methods please contact Flinders Technologies Pty. Ltd. for license information. flinderstech@flinders.edu.au

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