

SSP-SAP
TARGETED SAP CONJUGATE

*a tool for eliminating cells that express substance P receptor (NK-1);
targeted via Substance P, eliminated via saporin*

Catalog Number: IT-11
Quantity: 25 micrograms, 100 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:

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.

SSP-SAP is a conjugation of saporin and SSP, the Sar⁹, Met(O₂)¹¹ analog of Substance P. These two amino acid replacements are at two sites of digestion by tissue proteases (there is a third that is still in the analog, so it does eventually get degraded). Since the targeting agent is, of course, necessary for the cytotoxic activity, SSP-SAP will diffuse farther, and thus hit more target cells. This specific analog of SP-saporin resists peptidase digestion, allowing a greater diffusion from the injection site before its metabolism. SSP-SAP eliminates cells expressing the Substance P (NK-1) receptor. Behaviors associated with pain perception are greatly affected by the injection of SSP-SAP into the spinal cord of rats. It is not suitable for retrograde transport.

Specificity and Preparation:

This targeted toxin (molecular weight 33 kDa) recognizes substance P receptor-bearing neurons *in vivo*. SSP-SAP is a chemical conjugate of the peptidase-resistant [Sar⁹, Met(O₂)¹¹] analog of Substance P and the ribosome-inactivating protein, saporin (Cat. #PR-01). This product is routinely tested by cytotoxicity assay.

Usage and Storage:

SSP-SAP eliminates cells expressing the Substance P (NK-1) receptor. All other cells are left untouched, even when they are the predominant cell type. This specific analog of SP-saporin resists peptidase digestion, allowing a greater diffusion from the injection site before its metabolism. Not suitable for retrograde transport.

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.



SSP-SAP TARGETED SAP CONJUGATE

Available Control(s): Blank-SAP

References:

1. Wang H, Germanson TP, Guyenet PG (2002) Depressor and tachypneic responses to chemical stimulation of the ventral respiratory group are reduced by ablation of neurokinin-1 receptor-expressing neurons. *J Neurosci* 22(9): 3755-3764.
2. Martin JL, Sloviter RS (2001) Focal inhibitory interneuron loss and principal cell hyperexcitability in the rat hippocampus after microinjection of a neurotoxic conjugate of saporin and a peptidase-resistant analog of substance P. *Journal Comp Neurol* 436:127-152.
3. Wiley RG, Lappi DA (1999) Targeting neurokinin-1 receptor-expressing neurons with [Sar⁹, Met(O₂)¹¹] substance P-saporin. *Neurosci Lett* 277(1):1-4.

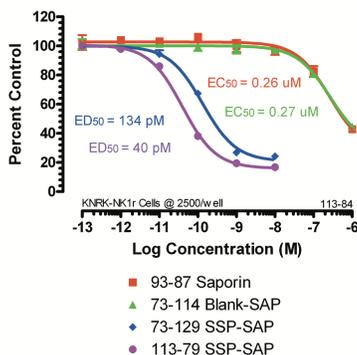
Safety:

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.

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KNRK-NK1r cells were plated at 2500 cells/well and incubated overnight. Saporin (Cat. #PR-01), SSP-SAP (Cat. #IT-11) and Blank-SAP (Cat. #IT-21) were added in 10 ul volumes and the plates left to incubate for 72 hours. PMS/MTS developing reagent was added and the plates read at 490 nm.

Limited Label License

This product is the subject of claims in a U.S. patent entitled "Substance P-Saporin (SP-SAP) Conjugates and Methods of Use Thereof" (6,063,758 and 7,741,435), owned by Advanced Targeting Systems, Inc. Purchase of this product conveys to the buyer only the non-transferable right under this patent to use the product in research conducted by the buyer. For information on purchasing a license to this product for purposes other than research, contact the Vice President of Business Development, Advanced Targeting Systems, 10451 Roselle St #300, San Diego, CA 92121. Phone: (858) 642-1988 Fax: (858) 642-1989.