



# Neurotensin-SAP TARGETED SAP CONJUGATE

a tool for eliminating cells that express neurotensin receptor; targeted via neurotensin, eliminated via saporin

**Catalog Number:** IT-56

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.

Neurotensin is a 13-amino-acid peptide found in the brain and spinal cord (first characterized by Carraway and Leeman). It is released by the hypothalamus and synthesized as part of a larger precursor protein that also includes the related neuropeptide neuromedin N. Neurotensin affects pituitary hormone release, interacts with the dopaminergic system, and is involved in vasodilation and hypotension. It can also modulate pain perception. Intrathecal neurotensin has also been shown to be anti-nociceptive. Neurotensin-SAP will specifically eliminate cells that express neurotensin receptors.

## **Specificity and Preparation:**

This targeted toxin recognizes cells that express neurotensin receptors. Neurotensin-SAP is a chemical conjugate of neurotensin and the ribosome-inactivating protein, saporin. This product is routinely tested by cytotoxicity assay.

#### **Usage and Storage:**

Neurotensin-SAP specifically eliminates neurotensin receptor-expressing cells. All other cells are left untouched. 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 <u>must</u> 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. The material can be handled safely using normal laboratory precautions.

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.



#### Neurotensin-SAP TARGETED SAP CONJUGATE

Available Control(s): Blank-SAP

#### **References:**

1. Wiley RG, Lappi DA (2010) Targeting Inhibitory Neurons in the Superficial Dorsal Horn: Neurotensin-Saporin (NTS-SAP) and Neurotensin-Cholera Toxin A Subunit (NTS-CTA) *Soc Neurosci Mtg*, *San Diego CA*, Abstract #585.2

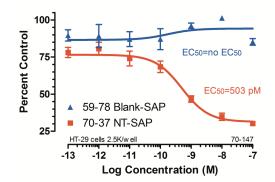
#### Safety:

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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HT-29 cells, a human epithelial colorectal adenocarcinoma cell line, were plated at 2500 cells/90 µl/well in a 96-well plate and incubated overnight. Blank-SAP and Neurotensin-SAP were then added in 10-µl volumes to each well. The plates were incubated 72 hours. Plates were developed with sulforhodamine B then read at 594 nm. Data analysis was done by PRISM (GraphPad).