

**NKB-SAP (NK3-SAP)**
TARGETED SAP CONJUGATE

*a tool for eliminating cells that express NK3 (NKB receptor);
targeted via neurokinin B peptide ([MePhe⁷]-NKB), eliminated via saporin*

Catalog Number: IT-63
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.

An analog of the Neurokinin B peptide, [MePhe⁷]-NKB,¹ is a selective agonist of the NK3 receptor.^{2,3} Conjugation of [MePhe⁷]-NKB to saporin (NK3-SAP) allows for the specific elimination of a subpopulation of neurons expressing kisspeptin, neurokinin B, and dynorphin (KNDy neurons) through their co-expression of the NK3 receptor.⁴ Although NK3 receptor expressing neurons reside in several locations, KNDy neurons reside only within the hypothalamic arcuate nucleus of many mammalian species and are critical for reproductive function. NK3-SAP can also be used to target and selectively destroy NK3r-expressing cells in other regions of the brain.

Specificity & Preparation: This targeted toxin recognizes cells expressing NK3 (NKB receptor). NKB-SAP (NK3-SAP) is a chemical conjugate of an analog of the neurokinin B peptide ([MePhe⁷]-NKB) and the ribosome-inactivating protein, saporin.

Usage: NKB-SAP specifically eliminates cells expressing NK3 (NKB receptor). All other cells are left untouched. **There may be lot-to-lot variation in material; working dilutions must be determined by end user.**

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.



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Selected References:

1. Drapeau G, d'Orléans-Juste P, Dion S, Rhaleb N-E, Regoli D (1987) Selective agonists for substance P and neurokinin receptors. *Neuropeptides* 10:43-54.
2. Drapeau G, d'Orléans-Juste P, Dion S, Rhaleb N-E, Regoli D (1987) Specific agonists for neurokinin B receptors. *Eur J Pharmacol* 136:401-403.
3. Corboz MR, Rivelli MA, Eckel SP (2010) Bronchoconstrictor effect of the tachykinin NK(3)-receptor agonists [MePhe(7)]-neurokinin B and senktide in the isolated guinea pig lung. *Exp Lung Res* 36:509-521.
4. Mittelman-Smith MA, Williams H, Krajewski-Hall SJ, Lai J, Ciofi P, McMullen NT, Rance NE. (2012) Arcuate kisspeptin/neurokinin B/dynorphin (KNDy) neurons mediate the estrogen suppression of gonadotropin secretion and body weight. *Endocrinology* 153(6):2800-2812.
5. Rance NE, Mittelman-Smith MA and Krajewski-Hall SJ (2012) Use of a novel saporin conjugate (NK3-SAP) to study the function of neurokinin 3 receptor (NK3r)-expressing kisspeptin/neurokinin B/dynorphin (KNDy) neurons in the rat arcuate nucleus. *Targeting Trends* 13(2):1,6.
6. Ogawa S, Nathan FM, Parhar IS. (2014) Habenular kisspeptin modulates fear in the zebrafish. *Proc Natl Acad Sci U S A* 111(10):3841-3846.

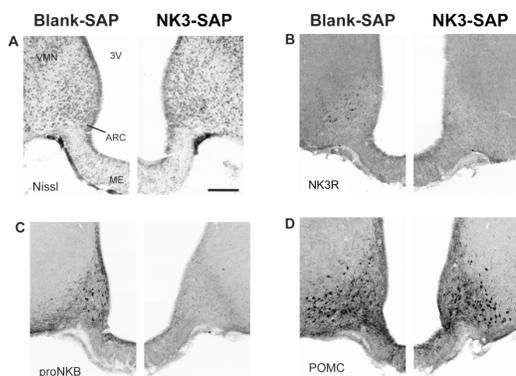
Control(s): Blank-SAP

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.

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Representative photomicrographs of sections from rats with injections of Blank-SAP (left) or NK3-SAP (right) in the arcuate nucleus, stained with Cresyl-violet (A) or immunohistochemistry (B-D). A: Cresyl violet-stained sections show preservation of the Nissl-architecture in NK3-SAP rats. B-C: NK3-SAP rats exhibited near total depletion of NK3r and proNKB immunoreactive neurons and fibers in the arcuate nucleus and median eminence. D: There was no difference in the number of proopiomelanocortin (POMC) neurons between Blank-SAP and NK3-SAP rats, demonstrating specificity of NK3-SAP.

Abbreviations: 3V, third ventricle; ARC, arcuate nucleus; ME, median eminence; NK3r, neurokinin B receptor; proNKB, proneurokinin B; VMN, ventromedial nucleus. Scale bar in A = 100 μ m and applies to all.

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