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Orexin-SAP
TARGETED TOXIN

[orexin-B peptide (hypocretin-2)]-saporin
targets orexin receptor

Catalog Number: IT-20
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 toxins 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 toxin is administered to the 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 which do not have the cell surface marker are not affected.

Specificity and Preparation:

This targeted toxin (molecular weight 33 kDa) recognizes cells that express orexin receptors. Orexin-SAP is a chemical conjugate of the 28 amino-acid orexin-B peptide (hypocretin-2) and the ribosome-inactivating protein, saporin. The orexin B sequence is the rat/mouse sequence.

Usage and Storage:

Orexin-SAP specifically eliminates cells expressing orexin receptors. 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 must assess the proper working dilution before beginning a full experimental protocol.**

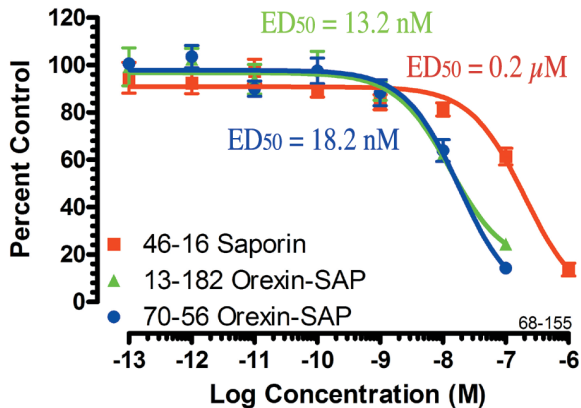
Centrifuge material at low speed in microfuge to ensure all of solution is at bottom of tube. Vortex gently. The material should be stored at -20°C in undiluted aliquots. Material should be aliquoted to a convenient volume and quantity to avoid repeated freezing and thawing that can damage the protein 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.

Available Control(s): Saporin, Blank-SAP

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HCT-116 cells were plated at 2500 cells/well/90 μ l in a 96-well plate and incubated overnight. Orexin-SAP dilutions were made in cell media and added in 10- μ l volumes. The plates were incubated 72 hours. PMS/MTS developing reagent was added and the plates were incubated 1 hour, then read at 490 nm in a plate reader. Data analysis was done by PRISM (GraphPad).

References:

1. Gerashchenko D, Kohls MD, Greco M, Waleh NS, Salin-Pascual R, Kilduff TS, Lappi DA, Shiromani PJ (2001) Hypocretin-2-saporin lesions of the lateral hypothalamus produce narcoleptic-like sleep behavior in the rat. *J Neurosci* 21(18):7273-7283.
2. Gerashchenko D, Salin-Pascual R, Shiromani PJ (2000) Effects of hypocretin-saporin injections into the medial septum on sleep and hippocampal theta. *Brain Res* 913:106-115.
3. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92:573-585.

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.