ADVANCED TARGETING SYSTEMS

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CCK-SAP

TARGETED SAP CONJUGATE

a tool for eliminating cells that express CCK Receptor; targeted via CCK, eliminated via saporin

Catalog Number: IT-31
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.
Cholecystokinin (CCK) is widely distributed in the central nervous system and the gastrointestinal tract. The 33-amino acid peptide contains a carboxyl terminal octapeptide sequence Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH2 which confers the biological activity of CCK, and where the tyrosine residue occurs in sulfated form. This octapeptide, CCK8(SO_3), has high affinity for the two structurally-defined CCK receptors types, CCK_1 and CCK_2. CCK-SAP recognizes and eliminates cells that express CCK Receptor. It is not suitable for retrograde transport.

Specificity and Preparation:
This targeted toxin (molecular weight 31 kDa) targets cells that express CCK Receptor. The toxin is a chemical conjugate of sulfonated CCK and the ribosome-inactivating protein, saporin (Cat. #PR-01).

Usage and Storage:
CCK-SAP specifically eliminates cells that recognize CCK Recector. 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.**
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.

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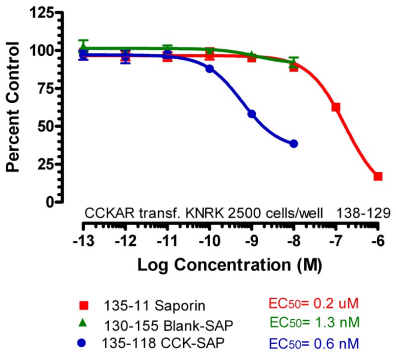
Available Control(s): Blank-SAP

References:
1. Zhang W, Gardell S, Zhang D, Xie JY, Agnes RS, Badghisi H, Hruby VJ, Rance N, Ossipov MH, Vanderah TW, Porreca F, Lai J (2008) Neuropathic pain is maintained by brainstem neurons co-expressing opioid and cholecystokinin receptors. *Brain* [Epub Dec 2].
2. Porecca F, Hruby V, Lai J (2003) Targeting Tests: CCK-SAP in binding studies. *Targeting Trends* 4(3):5.

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/support/protocols



KNRK cells transfected with the Cholecystokinin A receptor (CCKAR) were plated at 2,500 cells/90 µl/well in a 96 well plate and incubated overnight. CCK-SAP, Blank-SAP, and Saporin were added in 10 µl volumes and the plates were incubated for 72 hours. The plates were developed using a solution of XTT/PMS and read at 450 nm. Cytotoxicity was analyzed by comparing well readings of the treated wells to those of the control wells, expressed as a percentage. The number of viable cells remaining on the day of development is measured via cell metabolism of a colorimetric molecule within the developing reagents. Data analysis was performed using Prism software (GraphPad, San Diego).