Advanced Targeting Systems

101 STATE PLACE, STE L
ESCONDIDO, CA 92029 USA
01.858.642.1988 • WWW.ATSBIO.COM

Anti-vGAT-SAP

TARGETED SAP CONJUGATE

a tool for eliminating cells that express express the vesicular GABA transporter; targeted via affinity-purified anti-vGAT, eliminated via saporin

Catalog Number: IT-71
Quantity: 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.
Host: Rabbit

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.
Vesicular GABA transporters (vGAT) mediate the accumulation of GABA into synaptic vesicles and its release from nerve terminals. This transporter is expressed in the nerve endings of GABAergic neurons throughout the CNS and has also been found in the pancreas and pituitary gland. During development, expression of the vGAT protein changes. Expression can also change in response to patterns of neuronal activity.

Specificity & Preparation: Affinity-purified anti-vGAT was raised against the C-terminal of the rat vesicular GABA transporter.The antigen sequence is identical among human, rat, mouse, pig and guinea pig. Anti-vGAT-SAP is a bonded toxin between a biotinylated rabbit polyclonal antibody and the secondary conjugate Streptavidin-ZAP containing the ribosome-inactivating protein, saporin. This conjugate is routinely tested by cytotoxicity assays.

Usage: Anti-vGAT-SAP specifically eliminates cells that express the vesicular GABA transporter. It is useful in retrograde transport (see Wiley *et al*, 1989). **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.**

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.
For disposal: autoclave, or expose to 0.2 M NaOH, materials that come into contact with the toxin.

For research use only, not for use as a diagnostic.

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Selected References:

1. Wiley RG, Stirpe F, Thorpe P, Oeltmann TN (1989) Neuronotoxic effects of monoclonal anti-Thy 1 antibody (OX7) coupled to the ribosome inactivating protein, saporin, as studied by suicide transport experiments in the rat. *Brain Res* 505:44-54.

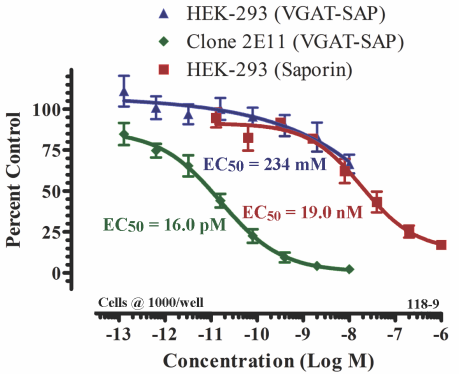
Control(s): BIgG-SAP rabbit

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/library/protocols



Cells were plated at 1000 cells/90 µl/well in a 96 well plate and incubated overnight. Anti-vGAT-SAP was added in 10 µl volumes and the plates were incubated for 72 hours. The plates were developed with SRB and read at 564 nm in a plate reader. Data analysis was done by PRISM (GraphPad, San Diego).

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