

192-IgG-SAP
TARGETED SAP CONJUGATE

*a tool for eliminating cells that express p75^{NTR} in rat;
targeted via the antibody to NGFR (192-IgG), eliminated via saporin*

Catalog Number: IT-01
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.
Host: Mouse

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.

Intraventricular injection of 192-IgG-SAP results in almost complete elimination of LNGFR (p75^{NTR})-positive cells in rat. 192-IgG-SAP is directed to a cell-surface antigen that is only expressed at high levels on neurons in the cholinergic basal forebrain (CBF). The antigen, p75^{NTR}, is not expressed on the neighboring, non-cholinergic neurons. 192-IgG-SAP specifically eliminates cholinergic neurons of the basal forebrain, medial septum, diagonal band of Broca, nucleus basalis of Meynert, and Purkinje neurons of the cerebellum. It provides researchers with a powerful lesioning tool - more specific and effective than chemical, surgical or electrolytic lesioning. Permanent and selective removal of cholinergic forebrain neurons makes an important animal model for the study of behavior, neuronal loss (e.g. Alzheimer's disease), plasticity of other systems in response to loss, replacement therapy, and drug effects and dependence.

Specificity and Preparation:

This targeted toxin recognizes p75^{NTR}-expressing cells in rat. 192-IgG-SAP is a chemical conjugate of the mouse monoclonal antibody to rat p75^{NTR} (Cat. #AB-N43) and the ribosome-inactivating protein, saporin (Cat. #PR-01). This product is routinely tested by cytotoxicity assay.

Usage and Storage:

192-IgG-SAP specifically eliminates cells expressing p75^{NTR}, also known as the low affinity nerve growth factor receptor. **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. 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.

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

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Available Control(s): Mouse IgG-SAP, Antibody to 192-IgG

References:

1. Birthelmer A, Dommes E, Jeltsch H, Cassel JC, Jackisch R (2002) Septal grafts and evoked acetylcholine release in the rat hippocampus after 192 IgG-saporin lesions. *Neuroreport* 13(7):973-976.
2. Ferencz I, Leanza G, Nonobashvili A, Kokaia Z, Kokaia M, Lindvall O (2001) Septal cholinergic neurons suppress seizure development in hippocampal kindling in rats: comparison with noradrenergic neurons. *Neurosci* 102(4):819-832.
3. Paqueron X, Li X, Eisenach JC (2001) p75-expressing elements are necessary for anti-allodynic effects of spinal clonidine and neostigmine. *Neurosci* 102(3):681-686.
4. Milner TA, Hammel JR, Ghorbani TT, Wiley RG, Pierce JP (1999) Septal cholinergic deafferentation of the dentate gyrus results in a loss of a subset of neuropeptide Y somata and an increase in synaptic area on remaining neuropeptide Y dendrites. *Brain Res* 831:322-336.
5. Pizzo DP, Waite JJ, Thal LJ, Winkler J (1999) Intraparenchymal infusions of 192 IgG-saporin: development of a method for selective and discrete lesioning of cholinergic basal forebrain nuclei. *J Neurosci Methods* 91:9-19.

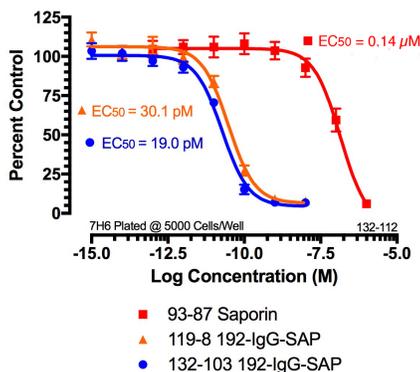
Safety:

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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7H6 cells were plated at 1000 cells/90 μl/well in a 96-well plate and incubated overnight. 192-IgG-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).