ADVANCED TARGETING SYSTEMS

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ME20.4-SAP

TARGETED SAP CONJUGATE

a tool for eliminating cells that express p75^{NTR} in multiple species;
targeted via NGFR monoclonal antibody (ME20.4), eliminated via saporin

Catalog Number: IT-15
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.
ME20.4-SAP provides researchers with a powerful lesioning tool — more specific and effective than chemical, surgical or electrolytic lesioning and is active in several species, including rabbit and primate. Intraventricular injection of ME20.4-SAP results in elimination of low affinity nerve growth factor receptor (p75^{NTR})-positive cells in rabbit. Tissue-directed injection in primates causes loss of p75^{NTR}-positive neurons. 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}-bearing cells in human, primate, rabbit, raccoon, dog, cat, pig and sheep. ME20.4-SAP is a chemical conjugate of the p75^{NTR} monoclonal antibody (Cat. #AB-N07) and the ribosome-inactivating protein, saporin (Cat. #PR-01). This product is routinely tested by cytotoxicity assay.

Usage and Storage:
ME20.4-SAP specifically eliminates p75^{NTR}-positive neurons. **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): Antibody to NGFr (p75) Mouse Monoclonal, Mouse IgG-SAP

References:

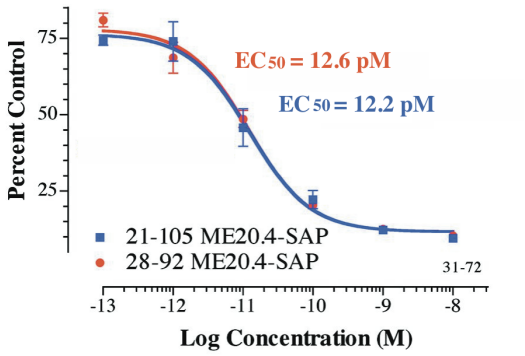
1. Ferreira G, Meurisse M, Tillet Y, Lévy F (2001) Distribution and co-localization of choline acetyltransferase and p75 neurotrophin receptors in the sheep basal forebrain: implications for the use of a specific cholinergic immunotoxin. *Neurosci* 104(2):419-439.
2. Barefoot HC, Baker HF, Ridley RM (2000) Synergistic effects of unilateral immunolesions of the cholinergic projections from the basal forebrain and contralateral ablations of the inferotemporal cortex and hippocampus in monkeys. *Neurosci* 98(2):243-251.
3. Beach TG, Potter PE, Kuo YM, Emmerling MR, Durham RA, Webster SD, Walker DG, Sue LI, Scott S, Layne KJ, Roher AE (2000) Cholinergic deafferentation of the rabbit cortex: a new animal model of Abeta deposition. *Neurosci Lett* 283:9-12.

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



HS294T cells are plated at 1000 cells/well and incubated overnight. ME20.4-SAP is added in 10 μl volumes and the plates are incubated 72 hours. PMS/MTS developing reagent is added and the plates are incubated 1-2 hours, then read at 490 nm.

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