

mu p75-SAP
TARGETED SAP CONJUGATE

*a tool for eliminating cells that express p75^{NTR} in mouse;
targeted via NGFR aff-pur rabbit polyclonal antibody, eliminated via saporin*

Catalog Number: IT-16
Quantity: 25 micrograms, 50 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: 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.

Conjugation of a low affinity nerve growth factor receptor (p75^{NTR}) antibody to saporin has produced a cytotoxin that eliminates the CBF neurons, while sparing neighboring neurons that express GAD, calbindin and parvalbumin. mu p75-SAP eliminates cells expressing p75^{NTR} in mouse. 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 mouse. mu p75-SAP is a chemical conjugate of the affinity-purified rabbit polyclonal antibody p75^{NTR} (Cat. #AB-N01AP) and the ribosome-inactivating protein, saporin (Cat. #PR-01). This product is routinely tested by cytotoxicity assay.

Usage and Storage:

mu p75-SAP specifically eliminates p75^{NTR}-positive cells. **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.

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): Antibody to NGFr (p75) affinity-purified rabbit polyclonal, Rabbit IgG-SAP

References:

1. Moreau PH, Cosquer B, Jeltsch H, Cassel JC, Mathis C (2008) Neuroanatomical and behavioral effects of a novel version of the cholinergic immunotoxin mu p75-saporin in mice. *Hippocampus* 18(6):610-622.
2. Ihunwo AO, Schliebs R (2007) BBeta-site amyloid precursor protein cleaving enzyme (BACE1) expression by astrocytes following p75-saporin immunolesion in transgenic TG2576 mice. *J Environ Neurosci Biomed* 1(1):41-77.
3. Hunter CL, Quintero EM, Gilstrap L, Bhat NR, Granholm AC (2004) Minocycline protects basal forebrain cholinergic neurons from mu p75-saporin immunotoxic lesioning. *Eur J Neurosci* 19(12):3305-3316.
4. Rossner S, Schliebs R, Bigl V (2000) Intracerebroventricular infusion of CHO5, a rat monoclonal antibody directed against mouse low-affinity nerve growth factor receptor (p75NTR), specifically labels basal forebrain cholinergic neurons in mouse brain. *Metab Brain Dis* 15(1):17-27.

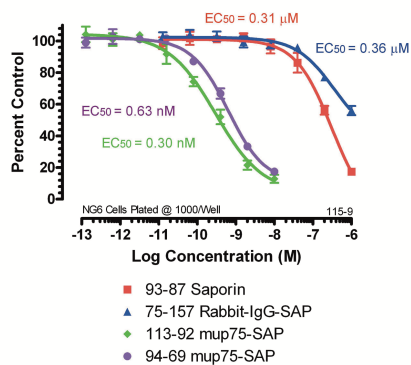
Safety:

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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NG6 cells are plated at 1000 cells/well and incubated overnight. Saporin, Rabbit-IgG-SAP (Cat. IT-35) and mu p75-SAP (conjugate of the affinity-purified rabbit polyclonal to mouse NGFr and saporin) are 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.