ADVANCED TARGETING SYSTEMS

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Mac-1-SAP rat

TARGETED SAP CONJUGATE

[antibody to mac-1 (CD11b)]-saporin
targets rat mac-1 (CD11b) receptor

Catalog Number: IT-33
Quantity: 25 micrograms, 100 micrograms, 250 micrograms
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.
CD11b is an alpha subunit of Mac-1, also known as CR3. CD11b is the receptor for the C3bi fragment of complement. This receptor is involved in bacterial phagocytosis. A reduction in neutrophil CD11b expression after severe traumatic injury correlates with increased septic complications. CD11b is a component of integrins, important for adhesion of neutrophils to surfaces. Mac-1-SAP recognizes the rat Mac-1 (CD11b) receptor in rat. Mac-1-SAP is excellent for removing contaminating macrophages from primary cultures to determine their role(s) in autoimmune diseases and in degenerative diseases such as Alzheimer's.

Specificity and Preparation:
This targeted toxin recognizes Mac-1-positive cells in rat. Mac-1-SAP is a chemical conjugate of the mouse monoclonal antibody to CD11b (the receptor for C3bi) and the ribosome-inactivating protein, saporin. The antibody was developed in mice immunized with rat neutrophils. It does not cross-react with the human or mouse epitope. The immunotoxin is extremely potent, three orders of magnitude more effective than non-targeted saporin and an ED50 of 6 pm in rat macrophages *in vitro*. This product is routinely tested by cytotoxicity assay.

Usage and Storage:
Mac-1-SAP eliminates Mac-1-positive (CD11b-positive) cells. All other cells are left untouched. **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): Antibody to Mac-1 Mouse Monoclonal (IgG_1), Mouse IgG-SAP

References:

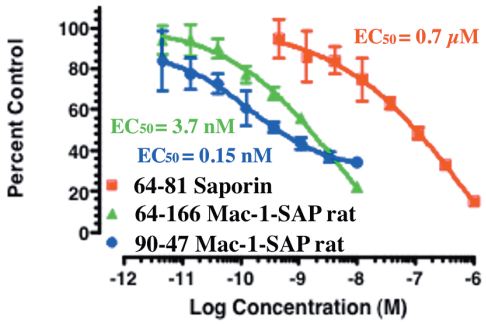
1. Kanold PO, Shatz CJ (2002) Developmental regulation of GABA receptor subunits requires subplate neurons. *Soc Neurosci Mtg, Orlando FL*, Abstract #530.11.
2. Sheehan JJ, Tsirka SE (2002) Reduction of microglia cell populations before induction of excitotoxicity reduces neurodegeneration. *Soc Neurosci Mtg, Orlando FL*, Abstract #606.9.
3. Kanai T, Watanabe M, Okazawa A, Sato T, Yamazaki M, Okamoto S, Ishii H, Totsuka T, Iiyama R, Okamoto R, Ikeda M, Kurimoto M, Takeda K, Akira S, and Hibi T (2001) Macrophage-derived IL-18-mediated intestinal inflammation in the murine model of Crohn's disease. *Gastroenterol* 121:875-888.

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/protocols



NR8383 cells, rat macrophages, are plated at 10000 cells/well and incubated overnight. Mac-1-SAP is added in 10 μl volumes and the plates are incubated 72 hours. Plates were developed using a sulforhodamine-B procedure, then read at 564 nm in a plate reader. Data analysis was done by PRISM (GraphPad).

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