ADVANCED TARGETING SYSTEMS

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Mac-1-SAP mouse/human
TARGETED SAP CONJUGATE
a tool for eliminating cells that express Mac-1 (CD11b) receptor in mouse or human; targeted via the antibody to CR3 (CD11b), eliminated via saporin

Catalog Number: IT-06
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: Rat

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 Mac-1 (CD11b) receptor in mouse and human. 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 mouse and human. Mac-1-SAP is a chemical conjugate of the rat monoclonal antibody to CD11b (the receptor for C3bi, Cat #AB-N05) and the ribosome-inactivating protein, saporin (Cat. #PR-01). The antibody is from the well-characterized M1/70 clone, and has been created by immunization from mouse macrophages, but reacts well with the human epitope also. It does not recognize the rat form. The immunotoxin is extremely potent, with an ED50 of 5 pM for a mouse monocytic cell line, more than five orders of magnitude more toxic than non-targeted saporin.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.
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 Mac-1 Rat Monoclonal (IgG_{2b}), Rat IgG-SAP

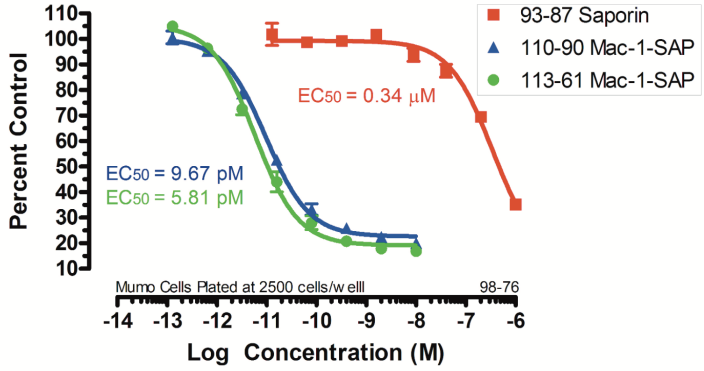
References:

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/support/protocols



MuMo cells, mouse monocytes, are plated at 2500 cells/well and incubated overnight. Reagents were added in 10 μl volumes at the indicated concentrations and incubated for 72 hours. XTT/PMS developing reagent was added and the plates were read on a Molecular Diagnostics Spectramax plate reader with SoftMax software. Data was analyzed by Prism 4.0 software.