

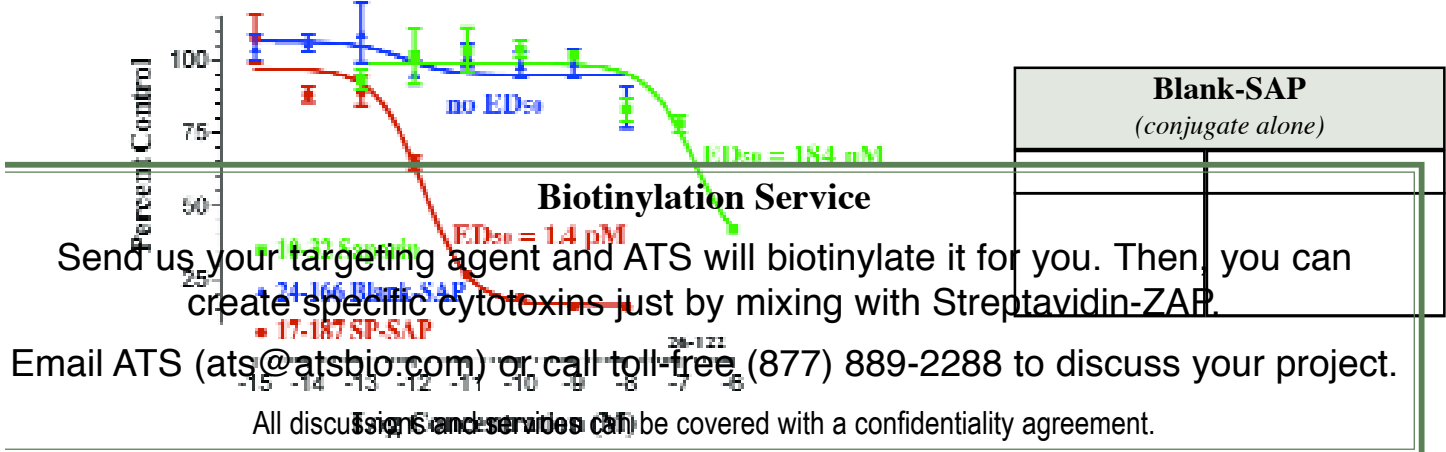


Control Conjugates

Blank-SAP

CONTROL FOR: peptide conjugates

a chemical conjugate between a non-targeted peptide and the ribosome-inactivating protein, saporin
 This molecule is the perfect control for use with peptide conjugates such as: SP-SAP, SSP-SAP, orexin-SAP, dermorphin-SAP, CRF-SAP, NPY-SAP, CCK-SAP, and Galanin-SAP. The sequence of the non-targeted peptide of Blank-SAP is an 11-amino acid, randomly mixed version of the sequence of melanocyte-stimulating hormone that is typical of peptides that bind to G-protein-coupled receptors. Examination of the peptide sequence using ENTREZ reveals no homologous sequences. Thus, Blank-SAP can be used as control for any non-specific effects of the toxin and provides a definitive baseline for determining the effects of a targeted peptide conjugate.



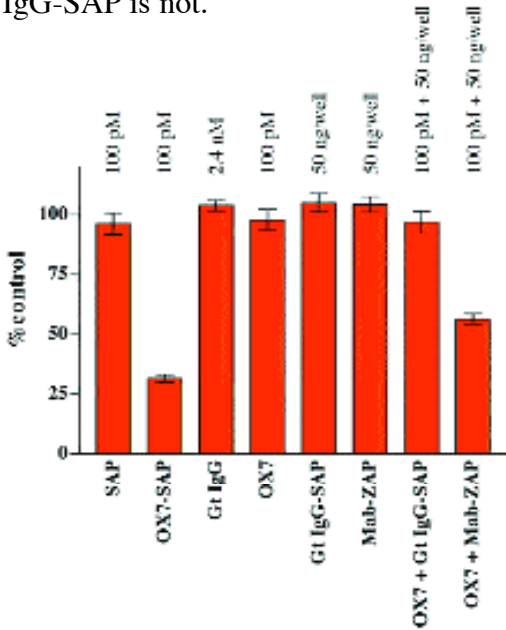
KNRK cells expressing the substance P receptor (NK-1r) were plated at 2500 cells/well in a 96-well plate and incubated overnight at 37°C. The following morning, SP-SAP, unconjugated saporin, or Blank-SAP was added. Plates were incubated for 3 days before being developed with MTS/PMS (Promega). The ED₅₀'s show that Blank-SAP has almost no activity in the tested concentration range.

Goat IgG-SAP

CONTROL FOR: secondary conjugates

a chemical conjugate of pre-immune goat IgG antibody and the ribosome-inactivating protein, saporin

This molecule is the perfect control for use with the secondary conjugates: Mab-ZAP (goat anti-mouse IgG conjugated to saporin), Rab-ZAP (goat anti-rabbit IgG conjugated to saporin), Hum-ZAP (goat anti-human IgG conjugated to saporin), Rat-ZAP (goat anti-rat IgG conjugated to saporin), and Anti-M-ZAP (affinity-purified goat anti-mouse IgM conjugated to saporin). The secondary conjugates are used with a primary antibody to determine if the primary antibody can internalize saporin and would, therefore, be suitable for conjugation as a primary immunotoxin. Goat IgG-SAP, used in place of the secondary conjugate, will give a definitive baseline for comparison of the activity of the primary antibody to internalize. The accompanying figure shows that, while the primary, in this case OX7, is able to internalize the secondary conjugate, the goat IgG-SAP is not.



Cytotoxicity of second immunotoxin and control immunotoxin Goat IgG-SAP to PC12 cells. Concentration of samples is above the bars for each. Samples are added to PC12 cells (5000 per well, put in culture the night before). Mab-ZAP and Goat IgG-SAP are at 50 ng/well, while the primary antibody is at 100 pM. The "primary" immunotoxin OX7-SAP (Cat. #IT-02) removes greater than 65% of the cells at 100 pM, and the second immunotoxin Mab-ZAP incubated with the OX7 antibody (Cat. #AB-N08) removes about 50%, while the control Goat IgG-SAP incubated with the OX7 antibody is not significantly different than untreated controls.

Goat IgG-SAP (conjugate alone)	
Cat #	Units
IT-19-25	25 micrograms
IT-19-100	100 micrograms
IT-19-250	250 micrograms

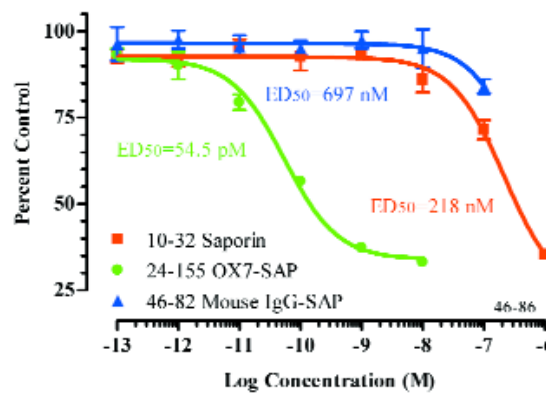
Mouse IgG-SAP

CONTROL FOR: mouse monoclonal conjugates

a chemical conjugate of pre-immune mouse IgG antibody and the ribosome-inactivating protein, saporin

This product serves as a control for the immunotoxins that use a mouse monoclonal: 192-IgG-SAP, OX7-SAP, Anti-DBH-SAP, ME20.4-SAP, Anti-SERT-SAP, Anti-CD25-SAP human, Mac-1-SAP rat. It is the same molecular weight, consists of comparable materials (saporin and a mouse IgG) and is synthesized with the same protocol as the targeted immunotoxins. The difference is the cell-specific antibodies are replaced with "blanks," antibodies that have no specificity, and no ability to target cells.

Mouse IgG-SAP (conjugate alone)	
Cat #	Units
IT-18-25	25 micrograms
IT-18-100	100 micrograms
IT-18-250	250 micrograms



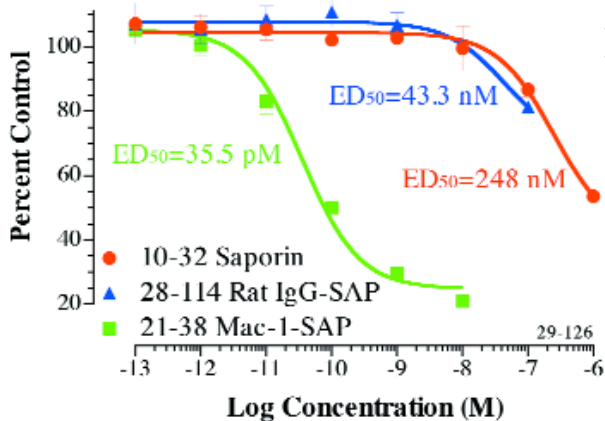
PC12 cells, a rat tumor cell line that expresses the Thy 1 receptor, were plated at 5000 cells per well and allowed to acclimatize overnight. The cells were exposed to the various reagents at the indicated concentrations for 72 hours. MTS/PMS (Promega) mixture was added and plates were read on a Molecular Diagnostics Spectramax plate reader with SoftMax software. Data was analyzed by Prism 4.0 software.

Rat IgG-SAP

CONTROL FOR: rat monoclonal conjugates

a chemical conjugate of pre-immune rat IgG antibody and the ribosome-inactivating protein, saporin

This product serves as a control for the immunotoxins that use a rat monoclonal: Mac-1-SAP mouse/human and Anti-DAT-SAP. It is the same molecular weight, consists of comparable materials (saporin and a rat IgG) and is synthesized with the same protocol as the targeted immunotoxins. The difference is the cell-specific antibodies are replaced with "blanks," antibodies that have no specificity, and no ability to target cells.



WEHI-274.1 cells, a murine monocytic cell line, were plated at 2500 cells per well in 90 μ l of medium. After allowing acclimatization overnight, the cells were exposed to the various reagents at the indicated concentrations for 72 hours. MTS/PMS (Promega) was added and after two hours, plates were read at 492 nm on a Molecular Diagnostics Spectramax 340 plate reader with SoftMax software. Data were analyzed by Prism 3.0 software.

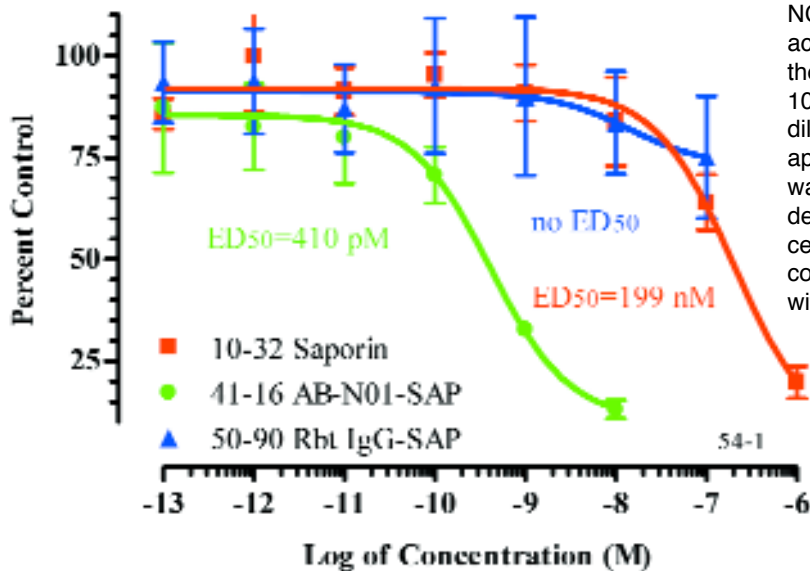
Rat IgG-SAP (conjugate alone)	
Cat #	Units
IT-17-25	25 micrograms
IT-17-100	100 micrograms
IT-17-250	250 micrograms

Rabbit IgG-SAP

CONTROL FOR: rabbit polyclonal conjugates

a chemical conjugate of pre-immune rabbit IgG antibody and the ribosome-inactivating protein, saporin

This product serves as a control for the immunotoxins that use a rabbit polyclonal: mu p75-SAP. It is the same molecular weight, consists of comparable materials (saporin and a rabbit IgG) and is synthesized with the same protocol as the targeted immunotoxins. The difference is the cell-specific antibodies are replaced with "blanks," antibodies that have no specificity, and no ability to target cells.



NG6 cells were plated at 1000 cells per well and allowed to acclimate for 15-18 hours. Reagents were added such that the most concentrated dose was 10 nM for AB-N01-SAP, 100 nM for Rabbit IgG-SAP, and 1 μ M for Saporin. 1:10 dilutions were made for a total of 8 dilutions at 6 replicates apiece. Plates were incubated at 37°C for 72 hours. ED₅₀ was determined by evaluating the percent of live cells, developed with a MTS/PMS mixture, and comparison to cells in wells that received no treatment. Data was collected on a Molecular Devices Spectramax plate reader with Softmax software, and evaluated with Prism software.

Rabbit IgG-SAP (conjugate alone)	
Cat #	Units
IT-35-25	25 micrograms
IT-35-100	100 micrograms
IT-35-250	250 micrograms