ADVANCED TARGETING SYSTEMS

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Human IgG-SAP

NON-TARGETED SAPORIN CONTROL MOLECULE

a tool for use as control for human IgG-containing immunolesioning agents; non-targeted via pre-immune human IgG antibody conjugated to saporin

Catalog Number: IT-49
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: Human

Background:
Controls are a vital part of the scientific procedure; without them it is difficult to isolate the specific effects from the non-specific or artifactual. This control molecule is the same molecular weight, consists of similar, comparable materials and is synthesized with the same protocols as the targeted conjugates. The difference is the cell-specific targeting agents are replaced with "blanks," antibodies or peptides that have no specificity, and no ability to target cells. In short, they are the perfect control molecules for behavioral experiments with Advanced Targeting Systems' targeted conjugates.
Human IgG-SAP serves as a control for the human IgG-containing immunolesioning agents. Conjugates utilizing human or humanized antibodies will benefit from using Human IgG-SAP as it is manufactured in an identical manner as other targeted conjugates, to give a definitive baseline for comparison.

Specificity and Preparation:
This control conjugate has no known specificity; it may react with cells that possess Fc receptors. Human IgG-SAP is a chemical conjugate of pre-immune human IgG antibody and the ribosome-inactivating protein, saporin. The product is routinely tested by cytotoxicity assay.

Usage and Storage:
Human IgG-SAP serves as a control for human IgG-containing immunolesioning agents. **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. The material should be stored at -20°C in undiluted aliquots. 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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References:

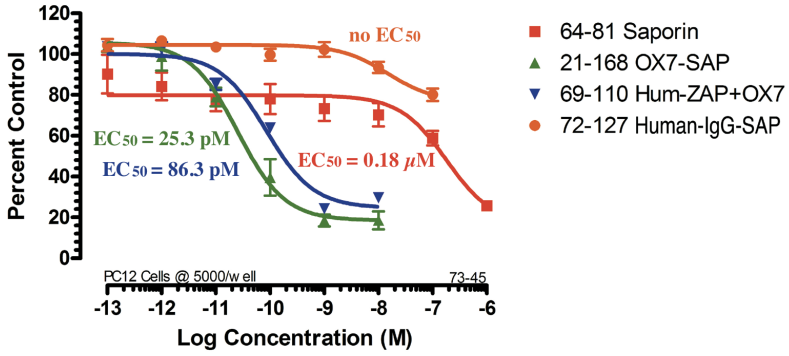
1. Tazzari PL, Bolognesi A, De Totero D, Falini B, Lemoli RM, Soria MR, Pileri S, Gobbi M, Stein H, Flenghi L, *et al*. (1992) Ber-H2 (anti-CD30)-saporin immunotoxin: a new tool for the treatment of Hodgkin's disease and CD30+ lymphoma: *in vitro* evaluation. *Brit J Haemat* 81:203-211.
2. Dinota A, Tazzari PL, Michieli M, Visani G, Gobbi M, Bontadini A, Tassi C, Fanin R, Damiani D, Grandi M, *et al*. (1990) *In vitro* bone marrow purging of multidrug-resistant cells with a mouse monoclonal antibody directed against Mr 170,000 glycoprotein and a saporin-conjugated anti-mouse antibody. *Cancer Res* 50:4291-4294.
3. Thorpe PE, Brown AN, Bremner JA Jr, Foxwell BM, Stirpe F (1985) An immunotoxin composed of monoclonal anti-Thy 1.1 antibody and a ribosome-inactivating protein from *Saponaria officinalis*: potent antitumor effects *in vitro* and *in vivo*. *J Natl Cancer Inst* 75(1):151-159.

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



PC12 cells were plated at 5,000 cells/90 µl/well and incubated overnight. Saporin, OX7-SAP, and Human IgG-SAP dilutions were made in cell media, and 10 µl was added to each well. OX7 antibody (AB-N08) was diluted in cell media containing, at a final concentration, 100 ng/10 µl Hum-ZAP, and 10 µl was added to each well. The plates were incubated 72 hours. The medium was dumped off of the plate, and the cells were fixed with ice-cold 10% TCA for 1 hour at 4°C. The plate was washed 3 times with tap water and allowed to air dry. 50 µl of 0.4% sulfarhodamine B/1% acetic acid was added to each well and the plate was incubated for 30 minutes at room temperature. The plate was washed 3 times with 1% acetic acid and allowed to air dry. The dye was solubilized with 100 µl of 10 mM unbuffered tris base per well, with 5 minutes of gentle shaking. The plate was read at 564 nm, and data analysis was done with Prism software (GraphPad, San Diego).