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Rat-ZAP
SECONDARY CONJUGATE

[affinity-purified goat anti-rat IgG]-saporin
uses YOUR rat monoclonal antibody to target and eliminate cells

Catalog Number: IT-26
Quantity: 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: Goat

Background:

Rat-ZAP uses your primary rat monoclonal IgG antibody to target and eliminate cells that recognize your primary antibody. The secondary conjugate uses the secondary antibody (affinity-purified goat anti-rat IgG) to "piggyback" onto YOUR rat primary antibody. Rat-ZAP can be utilized for screening rat IgG antibodies for internalization and/or their suitability to make potent immunotoxins. When the *in vitro* results confirm the desired specificity, it is recommended that you order a custom conjugation of your antibody to saporin.

Specificity and Preparation:

This secondary conjugate recognizes YOUR rat monoclonal antibody. Rat-ZAP is a chemical conjugate of affinity-purified goat anti-rat IgG and the ribosome-inactivating protein, saporin. This product is routinely tested by cytotoxicity assay.

Usage and Storage:

Rat-ZAP uses your rat primary antibody to target and eliminate cells. This secondary conjugate is used to evaluate the potential of a primary antibody to internalize. **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.

Do not use a reducing agent (such as dithiothreitol, beta-mercaptoethanol or ascorbic acid) with this material. It will inactivate the toxin.

If the primary antibody recognizes a human receptor the conjugate will be toxic to human cells

expressing the appropriate receptor. 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.

Note: When used in a cytotoxicity assay, un-bound primary antibody will compete with primary antibody bound to Rat-ZAP and may reduce cytotoxicity through competitive inhibition of the primary antibody-secondary conjugate complex.



Rat-ZAP SECONDARY CONJUGATE

Available Control(s): Goat IgG-SAP

References:

1. Kohls MD and Lappi DA. (2000) Mab-ZAP: A tool for evaluating antibody efficacy for use in an immunotoxin. *BioTechniques* 28(1):162-165.

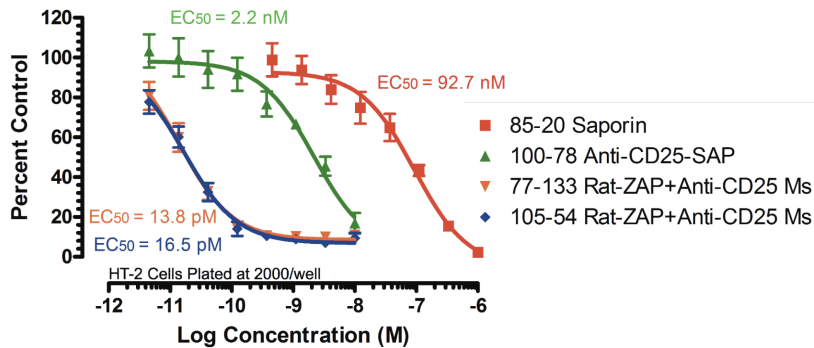
Safety:

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



HT-2 cells were plated at 2000 cells/90 μ l/well and incubated overnight. Saporin, and Anti-CD25-SAP dilutions were made in cell media, and 10 μ l was added to each well. Anti-CD25 antibody (Cat. #AB-19) was diluted in cell media containing, at a final concentration, 50 ng/10 μ l Rat-ZAP, and 10 μ l was added to each well. The plates were incubated 72 hours. Percent of live cells was evaluated by developing 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.