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Hum-ZAP SECONDARY CONJUGATE

*[affinity-purified goat anti-human IgG]-saporin
targets YOUR human monoclonal antibody*

Catalog Number: IT-22
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: Goat

Background:

Secondary conjugates are conjugations of a secondary antibody to the ribosome-inactivating protein, saporin (from the seeds of the plant, *Saponaria officinalis*). The secondary conjugate uses the secondary antibody to "piggyback" onto YOUR primary antibody in order to evaluate the ability of the primary antibody to internalize. Once the conjugate is internalized, saporin breaks away from the targeting agent and inactivates the ribosomes, which causes protein inhibition and, ultimately, cell death. Potency may vary according to the specificity and affinity of YOUR antibody to ITS receptor. Secondary conjugates are most effective in determining specificity of your antibody and suitability for conjugation as a primary immunotoxin. 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 (molecular weight 210 kDa) recognizes YOUR human monoclonal antibody. Hum-ZAP is a chemical conjugate of affinity-purified goat anti-human IgG and the ribosome-inactivating protein, saporin. This product is routinely tested by cytotoxicity assay.

Usage and Storage:

Hum-ZAP uses your human 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.**

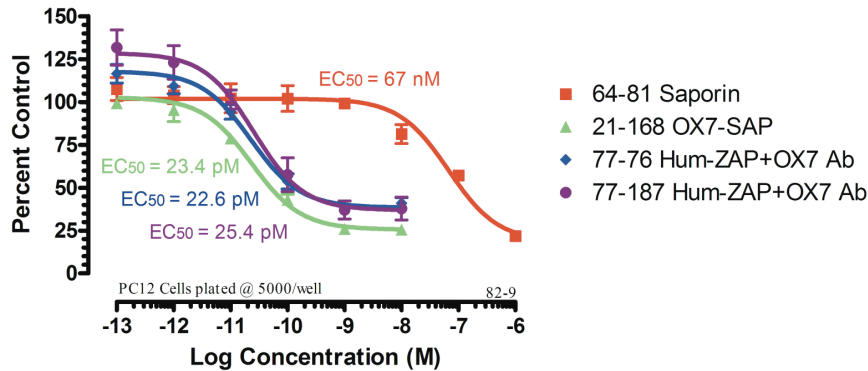
Centrifuge material at low speed in microfuge to ensure all of solution is at bottom of tube. Vortex gently. The material should be stored at -20°C in undiluted aliquots. Material should be aliquoted to a convenient volume and quantity to avoid repeated freezing and thawing that can damage the protein 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 Hum-ZAP and may reduce cytotoxicity through competitive inhibition of the primary antibody-secondary conjugate complex.

**Hum-ZAP
SECONDARY CONJUGATE**



PC12 cells were plated at 5000 cells/90 μ l/well and incubated overnight. Saporin and OX7-SAP dilutions were made in cell media, and 10 μ l was added to each well. OX7 antibody (AB-N08) was diluted in cell media containing, at a final concentration, 100 ng/10 μ l Hum-ZAP, and 10 μ l 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 μ l 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 μ l 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).

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.

Available Control(s):

Goat IgG-SAP

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.

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