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Rat-ZAP
SECONDARY CONJUGATE

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

Catalog Number: IT-26
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 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. The assay for eliminating targeted cells¹ uses the material listed below. There may be lot-to-lot variation in material; working dilutions must be determined by end user.

this secondary conjugate	your primary antibody
your cell line	medium
96 well plate (VWR #29442-054)	PMS (Sigma #P-9625, 5 g)
MTS (Promega #G1112, 240 mg)	

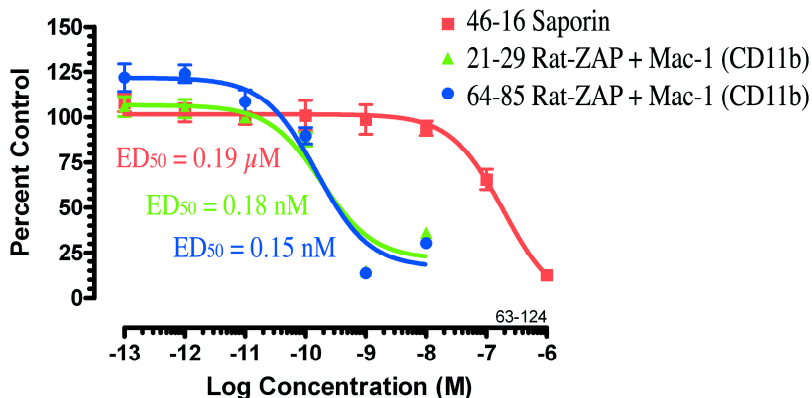
Centrifuge material at low speed in microfuge to ensure all of solution is at bottom of tube. Vortex gently. The material should be stored at -20°C in undiluted aliquots. Material should be aliquoted to a convenient volume and quantity to avoid repeated freezing and thawing that can damage the protein 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.

Available Control(s): Goat IgG-SAP

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WEHI-274.1 cells, mouse monocytes, were plated at 1000 cells/90 μ l/well in a 96-well plate and incubated overnight. Saporin and saporin conjugated to a rat monoclonal against human Mac-1 (CD11b) (Cat. #AB-N05) dilutions were made in cell media, and 10 μ l was added to each well. Rat anti-Mac-1 human (CD11b) was diluted in cell media containing, at a final concentration, 50 ng/10 μ l of Rat-ZAP, and 10 μ l was added to each well. The plates were incubated 72 hours and developed with MTS/PMS. Data was analyzed by Prism (GraphPad).

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.