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**Rab-ZAP**  
SECONDARY CONJUGATE

*[affinity-purified goat anti-rabbit IgG]-saporin  
targets YOUR rabbit polyclonal antibody*

**Catalog Number:** IT-05  
**Quantity:** 25 micrograms, 100 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 rabbit polyclonal antibody. Rab-ZAP is a chemical conjugate of affinity-purified goat anti-rabbit IgG and the ribosome-inactivating protein, saporin. This product is routinely tested by cytotoxicity assay.

**Usage and Storage:**

Rab-ZAP uses your rabbit 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.**

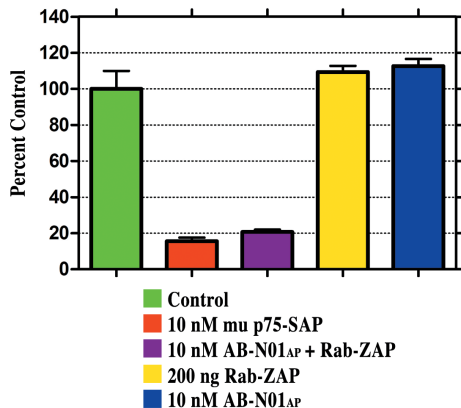
Centrifuge material at low speed in microfuge to ensure all of solution is at bottom of tube. Vortex gently. The material should be stored at -20°C in undiluted aliquots. Material should be aliquoted to a convenient volume and quantity to avoid repeated freezing and thawing that can damage the protein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

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**Activity of Rab-ZAP complexed with primary antibody to cells expressing primary antibody's antigen.**

In this experiment, cells expressing the antigen were challenged with a primary immunotoxin (mu p75-SAP, Cat. #IT-16). Cells were also challenged with the primary affinity-purified polyclonal antibody (AB-N01AP) at the same concentrations and pre-incubated with Rab-ZAP (Lot #69-30) at 200 ng per well. Both primary immunotoxin and Rab-ZAP have similar cytotoxicity to the target cells. Rab-ZAP alone at 200 ng per well has no effect, nor does the antibody alone at 10 nM.

**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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