

**Rab-ZAP**
ZAP CONJUGATE

*a tool to "piggyback" onto YOUR antibody via affinity-purified goat anti-rabbit IgG;
targeting cells that recognize YOUR primary rabbit affinity-purified polyclonal antibody, eliminated via
saporin*

Catalog Number: IT-05
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: Rab-ZAP uses your primary rabbit affinity-purified polyclonal IgG antibody to target and eliminate cells that recognize your primary antibody. This secondary conjugate uses the secondary antibody (affinity-purified goat anti-rabbit IgG) to "piggyback" onto YOUR rabbit primary antibody. Rab-ZAP can be utilized for screening rabbit IgG antibodies for internalization and/or their suitability to make potent immunotoxins.

Specificity & Preparation: This secondary conjugate recognizes YOUR rabbit affinity-purified 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: Rab-ZAP uses your rabbit affinity-purified polyclonal 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.**

Storage: Gently spin down material 5-10 seconds in a microfuge before use. 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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Selected References:

1. Ancheta LR, Shramm PA, Bouajram R, Higgins D, Lappi DA (2023) Streptavidin-saporin: Converting biotinylated materials into targeted toxins. *Toxins* 15(3):181. doi: 10.3390/toxins15030181
2. Kohls MD, Lappi DA (2000) Mab-ZAP: A tool for evaluating antibody efficacy for use in an immunotoxin. *BioTechniques* 28(1):162-165. doi: 10.2144/00281pf01 PMID: 10649788

Scan to view
all product
references.

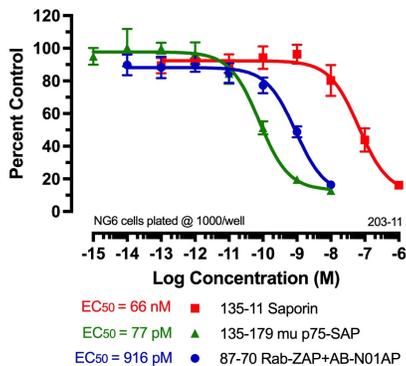
Control(s): Goat IgG-SAP

Safety:

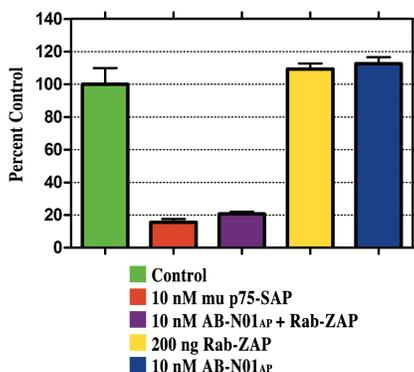
Good laboratory technique must be employed for safe handling of this product. This requires observation of the following practices:

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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NG6 cells were plated at 1000 cells/90 μ l/well and incubated overnight. Saporin, and mu p75-SAP dilutions were made in cell media, and 10 μ l was added to each well. NGFr (p75) antibody (AB-N01AP) was diluted in cell media containing, at a final concentration of 200 ng/10 μ l Rab-ZAP and 10 μ l was added to each well. The plates were incubated 72 hours. The plates were incubated for 72 hours. The plates were developed using a solution of XTT/PMS and read at 450 nm. Cytotoxicity was analyzed by comparing well readings of the treated wells to those of the control wells, expressed as a percentage. The number of viable cells remaining on the day of development is measured via cell metabolism of a colorimetric molecule within the developing reagents. Analysis was performed using Prism software (GraphPad, San Diego).



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.