

FabFc-ZAP human SECONDARY CONJUGATE

[goat anti-human monovalent antibody]-saporin uses YOUR human monoclonal antibody to target and eliminate cells

Catalog Number: IT-65

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:

FabFc-ZAP uses your primary human monoclonal IgG antibody to target and eliminate cells that recognize your primary antibody. The secondary antibody used in the Fc-specific Fab-ZAP will react only with the Fc portion of the IgG heavy chain. The FabFc-ZAP products can be used in B-cell research with the confidence that cell death is primary antibody-mediated. 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 human monoclonal antibody. FabFc-ZAP is a chemical conjugate of goat anti-human monovalent antibody and the ribosome-inactivating protein, saporin. The antibodies used to make FabFc-ZAP human are affinity-purified polyclonal antibodies against the Fc portion of human IgG. The antibody used in this product will not cross-react across immunoglobulin classes and subclasses of the same species, nor will it react with surface immunoglobulins. This product is routinely tested by cytotoxicity assay.

Usage and Storage:

FabFc-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 <u>must</u> 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 Fab-ZAP and may reduce cytotoxicity through competitive inhibition of the primary antibody-secondary conjugate complex.



FabFc-ZAP human SECONDARY CONJUGATE

Available Control(s): The recommended control for use with this product would be a non-binding primary antibody, such as an isotype control, that mimics your primary antibody targeting agent. This control antibody should be used with FabFc-ZAP identically to the manner in which you test your primary antibody of interest. However, as a convenient alternative, we do offer Goat IgG-SAP (Cat #IT-19) for use as a control toxin. Goat IgG-SAP is a non-binding immunotoxin made from pre-immune, bivalent IgG attached to saporin.

References:

- 1. Russell B and Ancheta L (2013) FabFc-ZAP: A New Secondary Conjugate. Targeting Trends 14(2):7.
- 2. Kohls MD, Lappi DA (2000) Mab-ZAP: A tool for evaluating antibody efficacy for use in an immunotoxin. *BioTechniques* 28(1):162-165.

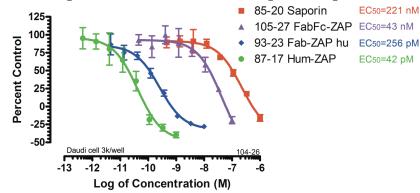
Safety:

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



This assay reflects non-specific killing only to demonstrate the necessity of using FabFc-ZAP (IT-65) when working with human B-cells due to endogenous cell-surface anchored IgG. No primary antibody was used. Efficacies of FabFc-ZAP and Fab-ZAP human are identical when used in the presence of primary antibody on non-B-cell human cell types. Daudi cells were plated at 3000 cells/90 μ l/well and incubated overnight. All dilutions were made in cell media, and $10~\mu$ l was added to each well. The plates were incubated 72 hours. The plates were developed using standard Sulforhodamine B protocol. The plate was read at 564 nm and data analysis was done with Prism software (GraphPad, San Diego).