ADVANCED
TARGETING
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Fab-ZAP mouse
ZAP CONJUGATE
a tool to "piggyback" onto YOUR antibody via goat anti-mouse monovalent antibody;
targeting cells that recognize YOUR mouse monoclonal antibody, eliminated via saporin

Catalog Number: IT-48
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:
Fab-ZAP uses your primary mouse monoclonal IgG antibody to target and eliminate cells that recognize your primary antibody. Fab-ZAP is made with a monovalent secondary antibody eliminating the possibility of cap formation, while preserving all the qualities that make an effective *in vitro* diagnostic tool. The antibodies used are affinity-purified polyclonal antibodies against both the heavy and light chain of mouse IgG. The antibody used in this product will cross-react across immunoglobulin classes and subclasses of the same species as they share the same light chain (either kappa or lambda). It also has an improved EC$_{50}$ when directly compared to Mab-ZAP in a cytotoxicity assay. Fab-ZAP can be utilized for screening mouse IgG antibodies for internalization and/or their suitability to make potent immunotoxins. 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 mouse monoclonal antibody. Fab-ZAP is a chemical conjugate of goat anti-mouse monovalent antibody and the ribosome-inactivating protein, saporin. The antibodies used to make Fab-ZAP mouse are affinity-purified polyclonal antibodies against both the heavy and light chain of mouse IgG. The antibody used in this product will cross-react across immunoglobulin classes and subclasses of the same species as they share the same light chain (either kappa or lambda). This product is routinely tested by cytotoxicity assay.

Usage and Storage:
Fab-ZAP uses your mouse 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.

For research use only, not for use as a diagnostic.   12/15/20

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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 Fab-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. 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/support/protocols
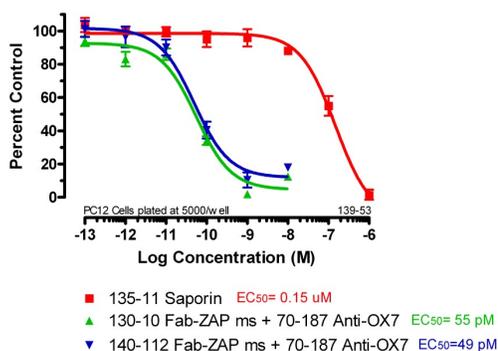
PC12 cells were plated at 5000 cells/90 μl/well and incubated overnight. Saporin dilutions were made in cell media and 10 μl was added to each well. OX7 antibody (AB-N08) was diluted in cell media containing, at a final concentration, 4.5 nM/10 μl Fab-ZAP, and 10 μl was added to each well. The plates 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).

- 135-11 Saporin   EC50= 0.15 uM
- 130-10 Fab-ZAP ms + 70-187 Anti-OX7   EC50= 55 pM
- 140-112 Fab-ZAP ms + 70-187 Anti-OX7   EC50=49 pM

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