ADVANCED TARGETING SYSTEMS
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ZAP Antibody Internalization Kit

INTRODUCTION

Screening large numbers of antibodies can be expensive in both cost and time. The ability to screen your antibody prior to direct conjugation is a great cost-benefit in the development of an effective targeted conjugate. Targeted conjugates are widely used to escort payloads to specific cell populations *in vitro* and *in vivo* for both basic research and pharmaceutical development. The development of an effective and specific targeted conjugate is a long and costly process. A molecule that targets the marker of choice (a Targeting Agent) must be identified, produced, and specificity must be characterized. Desirable traits of a Targeting Agent include high specificity and rapid internalization. The Targeting Agent can be an antibody, peptide, protein, or any other molecule that recognizes a cell-surface marker. Antibodies often make the best targeting agent, and the choice of the correct antibody is crucial to the specificity and performance of payload delivery.

The ZAP products produced by Advanced Targeting Systems consist of a variety of secondary antibodies that allow a large number of targeting agents to be screened quickly and cost-efficiently for specificity, functional binding, internalization, and EC_{50} determination. The wide selection of these secondary conjugates provide ideal tools for screening potential Targeting Agents. They are constructed using either species-specific secondary antibodies, or streptavidin (for use with biotinylated Targeting Agents), that are chemically attached to Saporin, the most potent of the plant ribosome-inactivating proteins. The mechanism of action of ZAP conjugates is detailed in Figure 1.

Use of a secondary conjugate eliminates the time-consuming and expensive step of conjugating each targeting-agent-candidate to the payload: the ZAP conjugate can simply be added to cells, in culture conditions, together with a Targeting Agent. Once the materials have been administered, the Targeting Agent takes the ZAP conjugate inside the cells of interest; the complex binds to a marker and the Saporin protein is released within the cytosol to inactivate the ribosomes. Cells that do not express the target cell surface marker do not bind or internalize the ZAP-targeting agent complex, and are not affected. Saporin has no binding chain, and no means of getting into cells on its own.

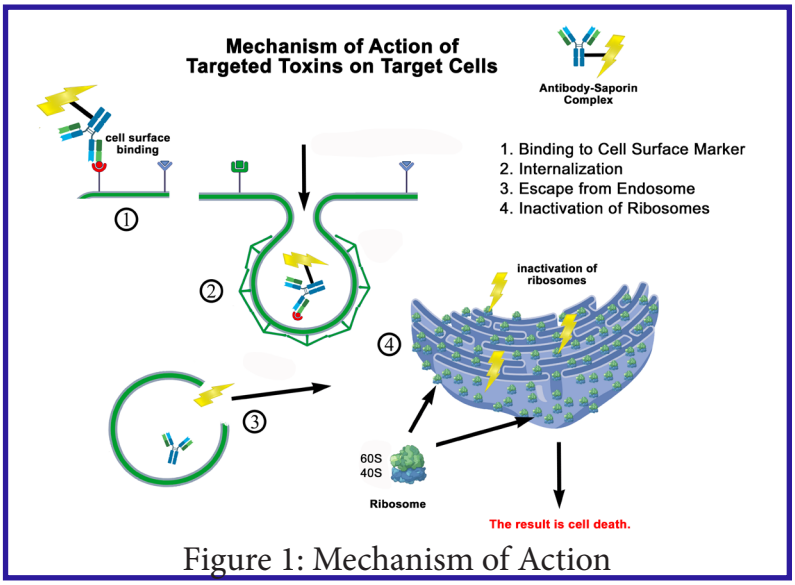

Figure 1: Mechanism of Action

Recommended protocols are available and are specific to the particular kit being used. Examples of predicted assay results are also included for comparison, and a successful assay provides an EC_{50} useful in determining if the candidate-targeting agent should be pursued at the next level.

KIT COMPONENTS

- Instruction booklet
- Flash drive with video tutorial
- ZAP Kit (see packing list for contents specific to your kit)

Provided by user:

- 96-well tissue culture treated, flat bottom plates with lids
- Cells expressing marker of interest
- Culture media for the cells
- Microcentrifuge tubes (1.5 ml size)

BACKGROUND AND GENERAL INFORMATION

Cell Culture

- Cells should be chosen that have significant levels of expression of the desired marker (either native, or transfected). Cell sorting using a flow cytometer can enrich a population of cells for the marker of interest.
- Cells are typically plated in the late afternoon (~16 hours prior to administration of the products). This is considered Day 1 of the assay.
- The number of cells per well are determined by the proliferation rate of the cells being used. Typical ranges include 1000-5000 cells per well of a 96-well plate, though as few as 100 cells and as many as 10,000 per well can be used in particular cases.
- The goal is to have the untreated cells approach 70-80% confluence on the morning of day five of the assay.

The ZAP Products

- Whole-ZAP products are made by conjugating species-specific polyclonal anti-IgG to Saporin. The secondary antibody used in the product is bivalent IgG against the whole IgG of the species chosen. As such, there will be some cross-reactivity to other immunoglobulin subtypes (IgA, IgM, IgE, etc.) due to light chain homology.
- Fab-ZAP products are made by using species-specific, polyclonal, monovalent, anti-IgG attached to Saporin. The antigen is against the whole IgG of the species chosen. As such, there will be some cross-reactivity to other immunoglobulin subtypes (IgA, IgM, IgE, etc.) due to light chain homology. The use of Fab IgG prevents the theoretical possibility of false-positive killing that could result from the capping phenomenon suffered by bivalent antibodies.
- FabFc-ZAP products also are manufactured using species-specific, polyclonal, monovalent, anti-IgG attached to Saporin. The difference is that the FabFc antibodies are made against only the Fc portion of the species-specific IgG. This means that the ZAP conjugate will not bind other Ig subclasses and will also not bind surface Ig that is typically expressed on B-cells.

BACKGROUND AND GENERAL INFORMATION (continued)

Saporin as Positive & Negative Control

- Saporin is a 30 kDa protein that cannot enter cells on its own, it has no binding chain. As a ribosome inactivating protein (RIP) it is only active and effective inside the cytosol of a cell.
- Using Saporin in the ZAP cytotoxicity assay is an essential control. Very high levels of saporin (≥1 μM) will cause non-specific death as a result of bulk-phase endocytosis by the treated cells. Using saporin in the assay starting at that concentration will ensure that the EC50 of non-specific killing is established.
- Testing multiple dilutions of Saporin not only establishes the EC50, but at lower concentrations of Saporin (typically <50 nM) there is not discernable elimination of the tested cell population. This titration of Saporin provides the baseline cell death profile by which the experimental targeting agents will be compared.
- It is recommended that the dilution profile for Saporin start at a higher concentration than the test samples, but follow a similar dilution schedule (i.e. 1:3 or 1:10).

Control-SAP

- The best possible control for use in this assay is a molecule that mimics the Targeting Agent candidate in every way but binding capability. A typical example would be an isotype control for an antibody candidate.
- As a worthwhile alternative when those options are not available, a species-specific non-binding control conjugate is recommended for use with the ZAP products. The antibodies used in these products come from normal serum of the species specified.
- Control conjugates should be used in the assay at the same concentration as the Targeting Agent candidate to demonstrate effects of a non-binding targeting agent at equivalent concentrations.

ADDITIONAL RESOURCES ON ATSBIO.COM

Protocols

Calculators

Videos

References