

# ZAP Biotin-Z Internalization Kit

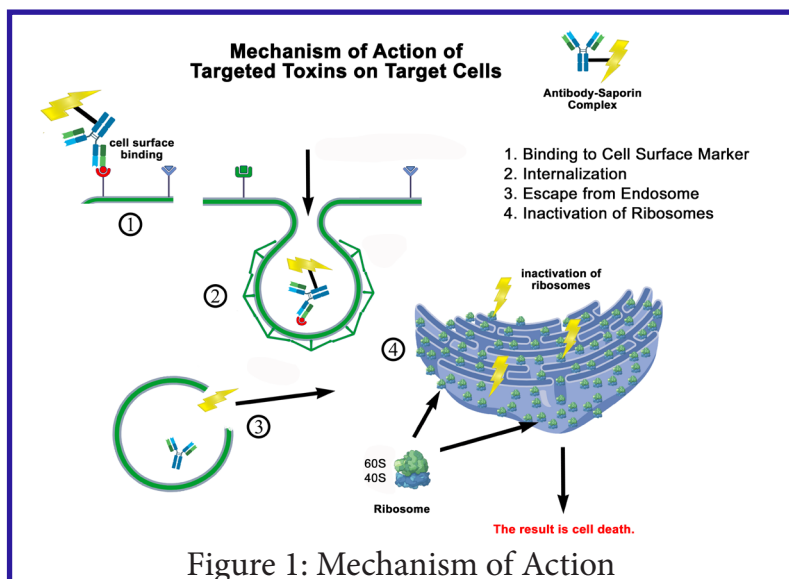
## INTRODUCTION

Screening large numbers of antibodies can be expensive in both cost and time. The ability to screen your biotinylated material 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<sub>50</sub> 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.

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<sub>50</sub> useful in determining if the candidate-targeting agent should be pursued at the next level.



# ZAP Biotin-Z Internalization Kit

## 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 Product

- Streptavidin-ZAP is a product uniquely designed for use with biotin-labeled molecules. The extremely strong interaction between streptavidin and biotin allows this product to also be used for initial *in vivo* studies. The streptavidin used in the product is in the tetramer form, so it is capable of binding up to four biotins per molecule of Streptavidin-ZAP. It is recommended that it used in an equimolar ratio with the targeting agent.

## 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 ( $\geq 1 \mu\text{M}$ ) 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  $< 50 \text{ nM}$ ) 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 non-binding control conjugate is recommended for use with the ZAP products. These control conjugates include a non-targeted peptide conjugate and non-targeted antibody conjugates 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