

Targeting Talk: Cytotoxicity Assays

by Dr. Douglas Lappi

One of the tests you can use to test your targeting agent for internalization is the *in vitro* Cytotoxicity Assay. Protocols to assist in preparing for, executing and interpreting results are now posted on our website. From the Home page (www.ATSbio.com) click on Protocols.

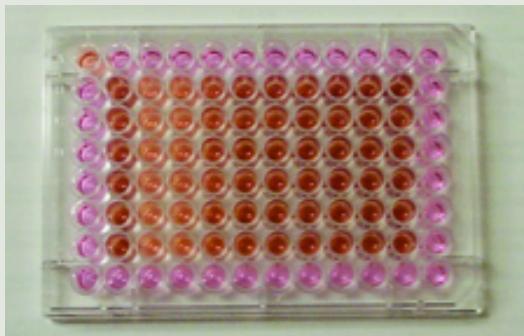
There are several protocols available.

1. Preparing for a Cytotoxicity Assay using Secondary Conjugates. This protocol will be helpful when using our secondary antibody-saporin conjugates with your primary antibody. These include Anti-M-ZAP (Cat. #IT-30), Goat-ZAP (Cat. #IT-36), Hum-ZAP (Cat. #IT-22), Mab-ZAP (Cat. #IT-04), Rab-ZAP (Cat. #IT-05), and Rat-ZAP (Cat. #IT-26).

2. Preparing for a Cytotoxicity Assay using Streptavidin-ZAP. This protocol will be helpful when using our streptavidin-saporin conjugate with your biotinylated targeting agent (peptide, ligand, cytokine, growth factor, antibody, etc.).

3. Concentration Calculation: Convert molarity to mg/ml and mg/ml to molarity. This protocol will help in determining the correct amount of material to use in your assay. There is also a link to an Online Calculator.

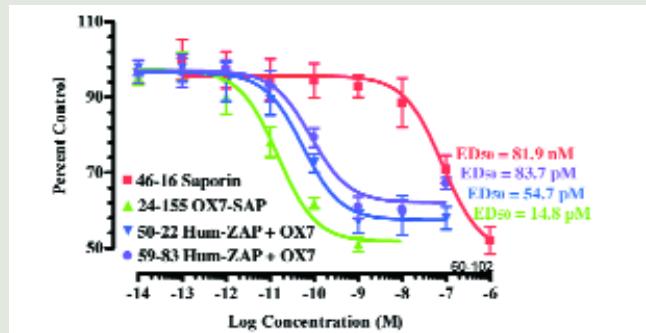
4. Cytotoxicity Assay for Targeted Toxins *in vitro*. This protocol includes photos of what your plates should look like during the assay process. It takes five days to complete this assay. Start on a Monday and develop on Friday. There are many factors that go into a successful



Day Five. Lighter-colored wells signify dead cells.
Your targeted toxin is working.

cytotoxicity assay. This protocol should help you design and execute appropriately.

5. Preparing Cytotoxicity Data. This protocol will give an example of how to process the data from a Cytotoxicity Assay. ATS uses SOFTMax Pro software connected to a plate reader to determine the A490 value. Then we import this data into Prism software (GraphPad) to conduct further data analysis. Here is a figure generated with Prism.



This graph gives important information about how the potency of your targeted toxin. The ED₅₀ is the Median Effective Dose (produces desired effect in 50% percent of population). The lower this number is, the more potent the targeted toxin.

We hope these protocols will be helpful to you in your research. If there are additional protocols or tutorials we can provide, please do not hesitate to ask.

Starting a new lab? Waiting for equipment?

Let us test your materials for you. ATS is expert at conducting *in vitro* assays with targeted toxins. Send us your primary antibody, peptide or protein, ligand, or lectin. When the *in vitro* results confirm the desired specificity, ATS can prepare a custom saporin conjugate.

Email ATS (ats@atsbio.com) or call toll-free (877) 889-2288 to discuss your project.

All discussions and services can be covered with a confidentiality agreement.