

# Targeting Talk: Cytotoxicity of Unbound Saporin

by Dr. Douglas Lappi

**Q:** We are re-examining some data collected using an immunotoxin not prepared by your company (VChAT-sap). Our results *in vivo* indicated that there were nonspecific effects although the creators claimed it was specific. We ran a Western Blot and determined that about half the saporin was not bound to the antibody. This may have been the problem but I want to confirm the cytotoxicity of unbound saporin. Can you confirm that? Further, the antibody in question never bound selectively in ferret tissue. Does this suggest a problem as well? Can you give me information on the best way to design and test an immunotoxin.

**A:** Yes, saporin at higher concentrations can be cytotoxic. Without specific binding, you will only see non-specific cytotoxicity. The sequence of the immunogen for that antibody is from the rat protein, so I'm not sure if it would target the ferret protein (there usually is good sequence homology among transporters). We worked a lot with this antibody and with an immunotoxin (made by us), but never got any sort of results that would indicate that it was working. We also were greatly concerned that the epitope is an intracellular epitope, and so we have difficulty understanding, from a theoretical standpoint, how it even could work. Because of many concerns, we never commercialized it, and we believe all the effects in the literature were non-specific, but "credible" because of the unusual experimental system that was used.

The best way to design and test an immunotoxin is to talk to us. If your antibody has been tested and shown to be internalized by the cell you are targeting, there should be no problem with the activity. The conjugate will only work as well as your antibody does.

We recommend that you try a second immunotoxin before having a custom conjugation performed. This allows you to use a secondary agent conjugated to saporin that "piggybacks" on your antibody and makes a second immunotoxin for use *in vitro* to test specificity and internalization of your antibody. You can check out the publication that talks about one of the secondary conjugates, Mab-ZAP. We also have Rab-ZAP that works with rabbit polyclonal antibodies, Rat-ZAP that works with rat antibodies, Hum-ZAP that works with human antibodies, anti-M-ZAP that works with IgM antibodies or you can biotinylate your material and use Streptavidin-ZAP.

You can find more information about second immunotoxins on our website. Click on Catalog:Targeted Toxins:Second Immunotoxins.

**REFERENCE:**

Kohls MD, Lappi DA (2000) Mab-ZAP: A tool for evaluating antibody efficacy for use in an immunotoxin. *BioTechniques* 28(1):162-165.

**Second Immunotoxins: Ready-Made Custom Conjugates**

IT-04	Mab-ZAP	Mouse Monoclonal antibodies	IT-26	Rat-ZAP	Rat Monoclonal antibodies
IT-05	Rab-ZAP	Rabbit Polyclonal antibodies	IT-30	Anti-M-ZAP	Mouse Monoclonal IgM antibodies
IT-22	Hum-ZAP	Human Monoclonal antibodies	IT-27	Streptavidin-ZAP	Biotinylated material

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