Targeting Tools: New Products

Controls for Immunotoxins

Advanced Targeting Systems announces two new control molecules for use with immunotoxins. We now offer mouse IgG or rat IgG conjugated to saporin. These new controls are the same molecular weight, consist of similar, comparable materials — saporin and a rat or mouse IgG — and are synthesized with the same protocols as the targeted immunotoxins. The difference is the cell-specific antibodies are replaced with "blanks," antibodies that have no specificity, and no ability to target cells. In short, they are the perfect control molecules for behavioral experiments with Advanced Targeting Systems' immunotoxins.

Controls are a vital part of the scientific procedure; without them it is difficult to isolate the specific effects from the non-specific or artifactual. With targeted toxin research, the same is true, and Advanced Targeting Systems often receives questions as to what makes the best control for a targeted toxin.

In the past, the response has been given according to what has been available. For an immunotoxin (a conjugate between an antibody and saporin), the suggested control is a mixture of the two components in their non-conjugated form. Of course, the lack of the conjugation process may detract from using this as a control, and there is always the question of how much antibody to mix with how much saporin.

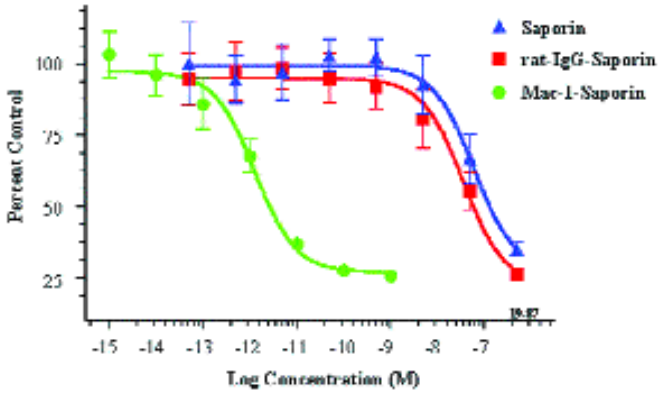
Another suggestion for a control, since often saporin is connected to its targeting agent via a disulfide bond, is to reduce the disulfide bond. This method has some difficulties: 1) the reducing agent, if not removed, can have its own effect, and 2) usually the process is incomplete (unless carried out under drastic conditions), leaving a percentage of active material in the control. Finally, it's expensive.

The new control immunotoxins avoid all of these difficulties. First of all, they are synthesized using the identical procedures that are used to synthesize the targeted immunotoxins, so there is no difference from the chemical point-of-view. They are very easy to use: they have the same molecular weight, you just use equal amounts of the control immunotoxin and the targeted immunotoxin. There are no complicated calculations to make. They are cost-effective. They are reasonably priced and time-saving because of the ease of preparation. As with all of the targeted immunotoxins, they are sterile-filtered and ready to go in phosphate-buffered saline at physiological pH.

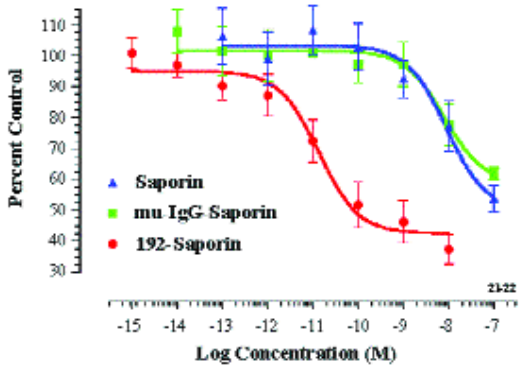
Mouse IgG-SAP(Cat# IT-18) serves as a control for immunotoxins that use a mouse monoclonal
192-Saporin (Cat# IT-01) Anti-DBH-SAP (Cat# IT-03) ME20.4-SAP (Cat# IT-15) OX-7-SAP (Cat# IT-02)
Rat IgG-SAP (Cat# IT-17) serves as a control for immunotoxins that use a rat monoclonal
Mac-1-SAP (Cat# IT-06) murine p75-SAP (Cat# IT-16)

In vitro data in the displayed graphs show that the control immunotoxins have orders of magnitude less cytotoxicity than the targeted immunotoxins. Their low toxicities are similar to that of saporin (on a molar basis), which is only taken into cells by bulk phase endocytosis, as opposed to antibody-mediated or receptor-mediated endocytosis of the targeted immunotoxins. These new molecules will make getting definitive data much easier.

Coming Soon: a new control peptide-toxin, that will use a randomly-generated, nonsense peptide conjugated with saporin. It will be the perfect control for SP-SAP, dermorphin-SAP and SSP-SAP.



WEHI-274.1, a murine monocytic cell line
ED_{50} for Mac-1-SAP: 1.3 pM; saporin: 60 nM; rat IgG-SAP: 36 nM.



C6 glioma, a rat tumor cell line that expresses p75
ED_{50} for 192-Saporin: 13 pM; saporin: 8.8 nM; mouse IgG-SAP: 7.9 nM
(The latter two calculated ED_{50}'s are skewed because the dosages are not sufficiently high to give the bottom plateau)

In both figures, cells were plated at 2500 cells per well in 90 microliters of medium.
After allowing acclimatization overnight, the cells were exposed to the various reagents in 10 µl at the indicated concentrations for 72 hours.
MTS (according to supplier's protocol: Promega) was added and after two hours, plates were read at 492 nM on a Molecular Diagnostics Spectramax 340 plate reader with SoftMax software.

Data analyzed by Prism 3.0 software.