Targeting Talk

by Dr. Douglas Lappi

Secondary Conjugates

Q: I'm using your secondary conjugate Mab-ZAP and it's not killing my cells.

A: Are you following the protocol on the data sheet? It's described in detail in the article by Kohls *et al.*[1]

Q: No, I'm doing flow cytometry. I have 70,000 cells per well. I mix Mab-ZAP with my primary antibody and add it. When I count the cells, there is no decrease.

A: That's a lot of cells per well. We use between 500 and 2500 over a 72-hour period and then develop with MTS.

Q: My cells grow very slowly. I didn't see anything after 72 hours.

A: If your cells are slow-growing, you may want to wait a little longer to develop the assay, because the whole metabolism process is slowed. This is a weakness of the MTS system--you have to have a certain number of cells in the end in the control cells to get a decent reading on your plate reader.

In this case, you might want to try a more sensitive assay such as protein synthesis inhibition. You can use protein or DNA synthesis inhibition with radiolabeled leucine or thymidine.

1. Kohls MD, Lappi DA (2000) BioTechniques 28(1):162-165.

Reducing Agents

Q: I have a question about what solutions might be incompatible with theconjugated saporins. We have done an experiment where we injected a mixture of saporin conjugate (same batch we've used in previous studies here) and a cocktail of 5,7-dihydroxytryptamine and 6-hydroxydopamine (in 0.1% ascorbic acid) to try to deplete multiple neurotransmitters.

The way we did this was to prepare both solutions at double strength and to mix them immediately before loading the syringe and placing the injections. So the final solution has 0.05% ascorbate, 0.01 ug/ul saporin conjugate, and I think 6 ug/ul 5,7-DHT and 4 ug/ul 6-OHDA.

Anyway, we are doing the histology now and the cholinergic lesion didn't work. I'm wondering whether the ascorbic acid might have either damaged the conjugation of the saporin to the antibody, or have inactivated the saporin molecule itself somehow.

A: You have well-described what the problem is. A reducing agent will inactivate the toxin, and of, course, ascorbic acid is a potent reducing agent. We have now decided, because of your experience, to put a line in the data sheet to caution people. This is the first report of this happening in nearly 14 years of business, so it just had not been an issue. That was a lack of foresight on our part.