

Biotinylated materials (continued)

(continued from page 1)



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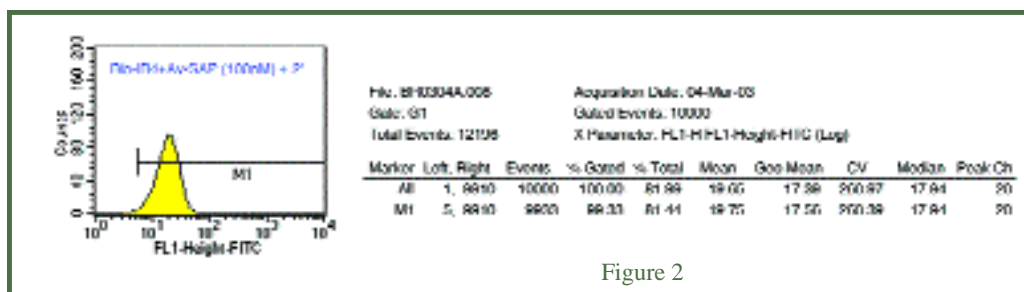


Figure 2

In Figure 2, IB4, a tetramer isolectin from *Bandeiraea simplicifolia*, was bound to biotin through an amide bond with a primary amine on the IB4 molecule. After incubation with the biotin, IB4 was run over a desalting column, and sample containing fractions determined by absorbance at 280 nm. A BCA was performed to determine protein concentration, and then binding evaluated through FACS analysis.

Paraformaldehyde-fixed KNRK cells, normal rat kidney cells positive for -D-galactose, were used for a FACS analysis with the biotinylated IB4 plus avidinylated-SAP (Cat. #IT-09). Cells were treated with biotinylated-IB4 and Avidinylated-SAP, both at a 100 nM concentration, and incubated for 1 hr. Cells were subsequently incubated with FITC-labeled anti-SAP (Cat. #FL-02) at a 1:50 dilution for 30 min. A 99% shift is seen as compared to the non-treated control.

In Figure 3, the biotinylated IB4 was combined with avidinylated-SAP (Cat #IT-09) and compared to the directly conjugated IB4-SAP (Cat # IT-10) in a cytotoxicity assay. KNRK cells were plated at 2500 per well in a 96-well plate and incubated overnight. IB4-SAP, biotinylated-IB4, avidinylated-SAP, and saporin were added in 10- μ l volumes, and the plates incubated 72 hours. The plates were developed with PMS/MTS for 1-2 hours, then read at 492 nm in a plate reader. Data analysis was done by PRISM (GraphPad, San Diego).

Results show cytotoxicity levels for the directly conjugated IB4-SAP are 4 times higher than for the biotinylated IB4/avidinylated-SAP. Cells targeted by biotinylated materials will be eliminated using avidinylated-SAP and this is a powerful and economical research tool for scientists in trying to determine the most specific targeting agent for their applications. Potency of avidinylated-SAP may vary according to the specificity and affinity of the biotinylated material to its receptor. ATS recommends the direct conjugation of your material to saporin when the *in vitro* results confirm the desired specificity.

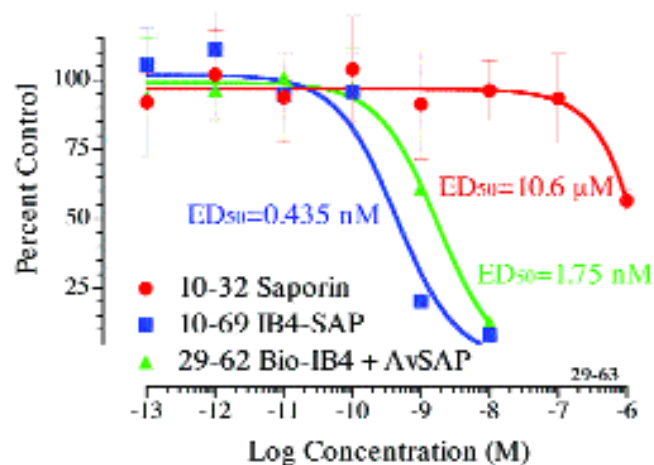


Figure 3

1. Vulchanova L, Olson TH, Stone LS, Riedl MS, Elde R, Honda CN. (2001) Cytotoxic targeting of isolectin IB4-binding sensory neurons. *Neuroscience* 108(1):143-155.

Targeting Teaser Winners



Congratulations to the puzzle solvers from our last newsletter. Each winner receives \$100 credit towards research product purchases from Advanced Targeting Systems.

The solution to the puzzle was:

Jumbles: WESTERN PROFILED MOLECULAR SPASTICITY INJECTION

Answer: What the student became when he tried to finish the chemistry experiment — A WEAPON OF "MASS DISRUPTION"!

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