## Volume 16, Issue 3

# Targeting Talk: Product Q&A

Q: For in vitro cytotoxicity assays, could you tell me:
1) whether you incubate primary with your Saporin secondary for a specific amount of time prior to cell addition, and

2) do you use a single concentration of secondary per well or a primary:secondary ratio -- like 1:2 or 1:4?

A: The primary antibody should be incubated with the ZAP product for 20 min prior to addition to the cells. Internalization often happens so quickly that you would lose some efficacy due to the antibody being bound and internalized <u>prior to</u> the ZAP product complexing with the primary.

We do recommend maintaining a constant 5 nM (~ 45 ng/well) concentration of the ZAP product in the well and titrating your primary only. This way the EC50 you generate will be the EC50 of the primary antibody with all else held constant. The best starting concentration for your primary antibody is 10-100 nM in the well.

- Q: Your targeted toxin kits come with different controls. I'm not sure of the best way to use them; there is included unconjugated antibody, unconjugated saporin, and a control conjugate, mouse IgG-SAP. Should I use them all in the same experiment or for different purposes?
- A: For mouse IgG-containing conjugates, the ideal control is Mouse IgG-SAP (Cat. # IT-18). Mouse IgG-SAP — that is, saporin conjugated to mouse IgG — that has no specific antigen for targeting is the best control. Unconjugated saporin is still considered a second good control, useful in cases where down-regulation by the antibody is a concern.
- Q: Which control is best to use with Octreotide-SAP?
- A: The best control to use with Octreotide-SAP is Blank-SAP (Cat. #IT-21). Blank-SAP serves as a control for all our peptide-targeted SAP conjugates. Listed below are the appropriate controls to use with our primary saporin conjugates.

Send a message on our website to get answers to your targeting questions.

#### (continued from page 3)

displayed significant deficits during the water maze task, indicating that GABAergic neurons in the medial septum are intrinsic to organization of spatial map-driven behavior.

## Exploratory behavior and recognition memory in medial septal electrolytic, neuro- and immunotoxic lesioned rats.

Dashniani MG, Burjanadze MA, Naneishvili TL, Chkhikvishvili NC, Beselia GV, Kruashvili LB, Pochkhidze NO, Chighladze MR.

#### Physiol Res Epub2015.

To investigate recognition memory that incorporates a spatial or temporal component, the authors lesioned the medial septum of rats using several techniques. For specific lesioning of cholinergic neurons rats received bilateral injections of 192-IgG-SAP (Cat. #IT-01, 500 ng total) into the medial septum. Saporin (Cat. #PR-01) was used as a control. While electrolytic lesions produced disruptions of spatial recognition memory, immunotoxin lesions did not, indicating that the cholinergic neurons of the septohippocampal pathway are not essential to processing this type of learning.

### **Appropriate Controls for Conjugates**

Blank-CTA (IT-61)	peptide-targeted CTA conjugates	SP-CTA and Neurotensin-CTA
Blank-SAP (IT-21)	peptide-targeted SAP conjugates	SSP-SAP, MOR-SAP, CRF-SAP, NPY-SAP, CCK-SAP, Galanin- SAP, Bombesin-SAP, Oxytocin- SAP, Neurotensin-SAP, NK3- SAP, Dyno-SAP and NMB-SAP
Fab IgG-SAP (IT-67)	goat IgG Fab-ZAP secondary conjugates	Fab-ZAP mouse, Fab-ZAP human Fab-ZAP rat, Fab-ZAP rabbit and FabFc-ZAP human
Goat IgG-SAP (IT-19)	goat IgG-containing targeted toxins	Mab-ZAP, Rab-ZAP, Hum-ZAP, Rat-ZAP, Anti-M-ZAP, Hug-M- ZAP and gPIG-ZAP
Human IgG-SAP (IT-49)	human IgG-containing targeted toxins	Custom Conjugates
Mouse IgG-SAP (IT-18)	mouse IgG-containing targeted toxins	192-IgG-SAP, OX7-SAP, Anti- DBH-SAP, ME20.4-SAP, Anti- SERT-SAP, Anti-CD25-SAP human, Mac-1-SAP rat, Anti- CD22-SAP, Anti-6 His-ZAP, Anti- GFP-ZAP, Anti-Basigin2-SAP, Anti-V5-ZAP, and Anti-FLAG (M5)-ZAP
Mouse IgM-SAP (IT-41)	mouse IgM-containing targeted toxins	Anti-M-ZAP
Rabbit IgG-SAP (IT-35)	rabbit IgG-containing targeted toxins	mu p75-SAP, GAT1-SAP, Goat- ZAP, Anti-ChAT-SAP, Melanopsin-SAP and Chick-ZAP
Rat IgG-SAP (IT-17)	rat IgG-containing targeted toxins	Mac-1-SAP, Anti-DAT-SAP, Anti CD25-SAP mouse and Anti- CD103-SAP