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**PREPARING FOR A CYTOTOXICITY ASSAY  
USING ATS SECONDARY ANTIBODY CONJUGATES**  
(Anti-M-ZAP, Goat-ZAP, Hum-ZAP, Mab-ZAP, Rab-ZAP, Rat-ZAP)

**GOAL:** To perform a Cytotoxicity Assay that will determine the ability of your targeting agent to  
1) bind to an extracellular marker, 2) internalize, and 3) cause cell death.

**HOW MUCH MATERIAL WILL YOU NEED?**

Targeting Agent: \_\_\_\_\_  $\mu\text{g}$   
(see "Concentration Calculation: Convert molarity to mg/ml and mg/ml to molarity")

Secondary Conjugate: \_\_\_\_\_  $\mu\text{g}$   
(see "Preparing Test Samples Using Secondary Conjugates")

Control Conjugate: \_\_\_\_\_  $\mu\text{g}$

**WHAT OTHER MATERIALS WILL YOU NEED?**

Cells that express target receptor (*Note: do not use reducing agent in media*)

Microcentrifuge tubes

96-well plates with lids

Media (appropriate for your cells)

MTS, Promega #G1111

PMS, Promega #G1112

**HOW LONG WILL THE ASSAY TAKE?**

Five days. (see "Cytotoxicity Assay for Targeted Toxins *in vitro*")

**WHAT EQUIPMENT/SOFTWARE WILL I NEED TO INTERPRET THE RESULTS?**

Vortexer

Incubator

Plate Reader

Softmax (Molecular Devices) or comparable software

Prism Software (Graphpad)

(see "Preparing and Interpreting Cytotoxicity Data")