

Use of Promega's CytoTox 96[®] Non-Radioactive Cytotoxicity Assay for the Study of MHC Class I Presentation of an Exogenous Antigen

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Editor's note: This feature highlights experiments abstracted from published, peer-reviewed journal articles in which Promega reagents and systems have contributed significantly to novel biological observations. The journal's publishers and authors have granted Promega permission to reproduce the figures shown here.

Brander, C. et al. (1993) *Carrier-mediated uptake and presentation of a major histocompatibility complex class I-restricted peptide.* Eur. J. Immunol. **23**, 3217.

In virus-infected cells, endogenous viral proteins are degraded in proteasomes and viral peptides are translocated to the endoplasmic reticulum (ER) (1,2). As the peptides traverse the ER, they become associated with MHC class I molecules and are co-transported to the cell surface by way of the Golgi apparatus (3,4). Antigen-specific CD8⁺ cytotoxic T lymphocytes (CTL) then can recognize the antigen in the context of the class I molecule, through the T cell receptor, and lyse the infected cell.

Processing and presentation of exogenous antigens occurs by a different pathway (5,6). The antigen is engulfed by endocytosis, degraded in endosomal compartments and presented on the cell surface in the context of MHC class II molecules. These antigen/class II complexes are recognized by CD4⁺ T cells.

In the featured paper, the authors reported achieving MHC class I presentation of an exogenous antigen by coupling the HLA-A2-restricted antigenic peptide from the influenza A virus matrix protein (residues 57-68, referred to as flu) to transferrin (Tf), which served as a carrier molecule. The carrier molecule allowed the Tf-flu to be taken up by cells via the transferrin receptor. Uptake and effective presentation by the target cell was detected in cytotoxicity assays using flu-specific, HLA-A2-restricted CD8⁺ CTLs. In all of the experiments, the target cells were incubated with either flu or Tf-flu and antigen presentation by the target cells was evaluated using Promega's CytoTox 96[®] Non-Radioactive Cytotoxicity Assay.

The CytoTox 96[®] Assay is a colorimetric alternative to ⁵¹Cr release cytotoxicity assays. This assay quantitatively measures the level of lactate dehydrogenase (LDH) that is released from lysed cells in much the same way as ⁵¹Cr release is quantitated in radioactive assays. The authors found the CytoTox 96[®] Assay to be as sensitive as the ⁵¹Cr release assay.

In [Figure 1](#), it was demonstrated that presentation of Tf-flu is restricted to the MHC class I pathway by the failure of this antigen to sensitize HLA-A2⁻ cells for CTL lysis. This conclusion was strengthened by the observation that Tf-flu-sensitized T2 target cells, which express only HLA-A2 (7,8), were killed by flu-specific CTLs ([Figure 2](#)).

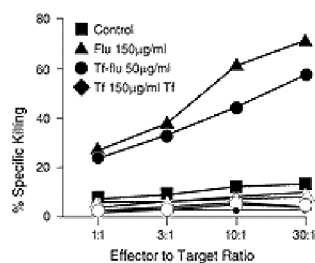


Figure 1. T cell-mediated cytotoxicity of flu-presenting target cells. B-LCL from a HLA-A2⁺ donor (closed symbols) and from a HLA-A2⁻ donor (open symbols) were incubated with flu, Tf-flu, Tf or medium alone for 90 minutes in culture medium at 37°C. After 4 hours, killing by a flu-specific CTL line was measured as LDH release in 50µl/well of supernatant as described in the original paper.

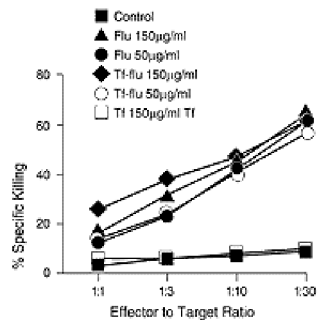


Figure 2. T cell-mediated cytotoxicity of flu- and Tf-flu-labeled T2 cells. T2 target cells were incubated with flu, Tf-flu, Tf or medium alone for 90 minutes at 37°C. Cytotoxicity of the flu-specific and HLA-A2 restricted CTL line was measured as LDH release from target cells.

Two different approaches were used to eliminate the possibility that Tf-flu binds to the cells by a direct interaction between flu and the HLA-A2 molecule, rather than binding to the transferrin receptor and entering through the class I pathway. The authors first showed that HLA-A2⁺ and HLA-A2⁻ B-lymphoblastoid cell lines (B-LCL) bind ⁵⁹Fe-labeled Tf-flu equally well, and that soluble flu peptide had no effect on the uptake of ⁵⁹Fe-Tf-flu (data not shown). They further demonstrated that Tf-flu binds to the Tf receptor by showing that binding of ⁵⁹Fe-labeled Tf-flu could be blocked completely by Tf in a dose-dependent manner (Figure 3).

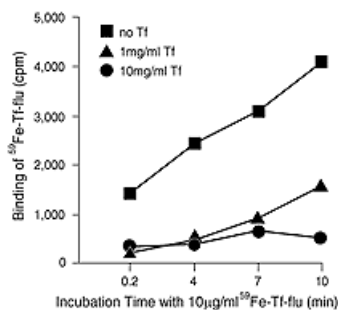


Figure 3. Inhibition of Tf-flu uptake by Tf on resting T cells. Resting T cells were isolated from a HLA-A2⁺ donor and incubated with ⁵⁹Fe-labeled Tf-flu (10mg/ml). Tf at the indicated concentration was added simultaneously at time 0. After different incubation times, unbound radioactivity was washed away and radioactivity in the cell pellets was measured using a gamma-counter.

The method of achieving MHC class I presentation described by Brander *et al.* is problematic in that the efficiency of uptake and presentation is relatively low. However, given that there are a number of potential applications for this procedure (e.g., investigating processing and presentation pathways in antigen presenting cells, and development of vaccines) additional experimentation to optimize this technology is warranted.

References

1. Monaco, J.J. (1992) *Immunol. Today* **13**, 173.
2. Yang, Y. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4928.
3. Townsend, A. *et al.* (1989) *Nature* **340**, 443.
4. Elliot, T. *et al.* (1991) *Nature* **351**, 402.
5. Christinck, E.R. *et al.* (1991) *Nature* **352**, 67.
6. Neefjes, J.J. and Momburg, F. (1993) *Curr. Opin. Immunol.* **5**, 27.
7. Hosken, N.A. and Bevan, M.J. (1990) *Science* **248**, 367.
8. Riberdy, J.M. and Cresswell, P. (1992) *J. Immunol.* **148**, 2586.

Ordering Information

Product	Size	Cat.#
CytoTox 96 [®] Non-Radioactive Cytotoxicity Assay	1,000 assays	G1780

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