

# Using Promega's CytoTox 96® Non-Radioactive Cytotoxicity Assay to Measure Cell Death Mediated by NMDA Receptor Subunits

Miroslav Cik, Paul L. Chazot, Sarah K. Coleman and F. Anne Stephenson

Pharmaceutical Chemistry Department,  
The School of Pharmacy, University of London,  
29/39 Brunswick Square, London WC1N 1AX

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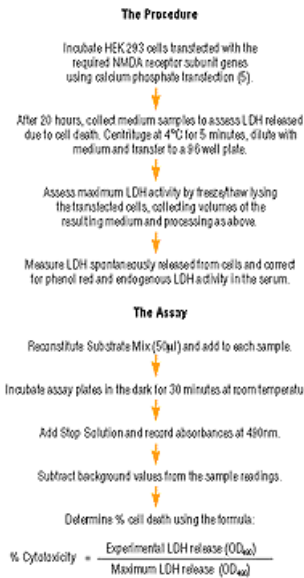
*In this brief report we describe the use of Promega's CytoTox 96® Non-Radioactive Cytotoxicity Assay to accurately and rapidly measure cell death mediated by transient expression of NMDA receptor subunits following calcium phosphate transfection of mammalian cells. This convenient method assesses cell death using medium alone and allows biochemical analysis of recombinant receptors on the same cells used for cytotoxicity measurements.*

Over-stimulation of excitatory l-glutamate transmission has been implicated in neurotoxic lesions causing neuronal loss associated with brain ischemic damage in stroke, epilepsy and hypoglycemia.

The N-methyl-D-aspartate (NMDA) receptors, a pharmacological subclass of glutamate receptors, are fast-acting, ligand-gated ion channels which are highly permeable for calcium. Excessive stimulation of NMDA receptors may overload intracellular calcium and may cause the activation of calcium-activated lipases and proteases, leading to cell death. Five genes, NMDA R1 and NMDA R2A-2D, (1) encode the subunits of the NMDA receptor, a heteromeric, integral membrane protein. In brain tissue, the subunits probably assemble as pentamers containing the NMDA R1 subunit with one or several of the R2 subunits.

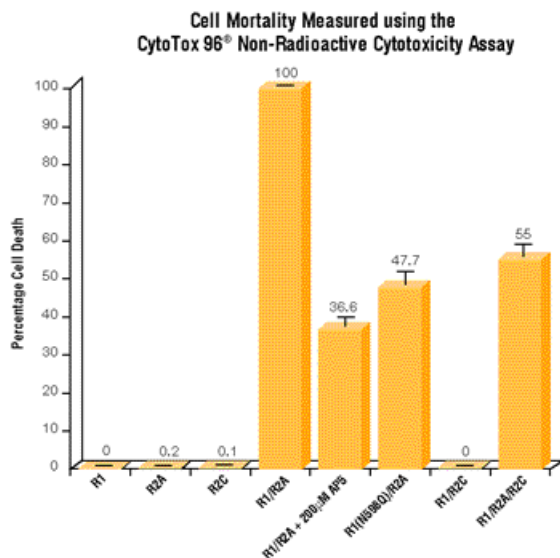
We expressed different NMDA receptor subunit combinations in HEK 293 cells to determine which subunits coexist in the native NMDA receptor of the brain and to study their respective properties. Co-expression of NMDA R1 and NMDA R2A subunits led to cell death following transfection (2). The inclusion of saturating concentrations of NMDA receptor antagonist in the medium, however, maintained cell viability, indicating that cell death, via activation of cloned and functional NMDA receptors was caused by l-glutamate in the medium. We demonstrated that the percentage cell death measured by Trypan Blue exclusion was equivalent to the cell transfection efficiency (data not shown).

In extending these studies to assess the role of different wild-type and mutant subunit combinations causing cell death, we found the Trypan Blue exclusion method laborious and of limited accuracy. To overcome these limitations, we have successfully applied Promega's CytoTox 96® Assay (3,4), which accurately and rapidly measures cell death by quantitating the release of lactate dehydrogenase (LDH), a stable cytosolic enzyme from lysed cells. Unlike the Trypan Blue assay, the CytoTox 96® Assay assesses cell death using medium alone and allows biochemical analysis of the recombinant receptors on the *same* cells used for the cytotoxicity measurements. The procedure is outlined in the flowchart in [Figure 1](#). [Figure 2](#) presents our results using the CytoTox 96® Non-Radioactive Cytotoxicity Assay to measure cell death mediated by transfected NMDA receptors.



1.

**Figure 1. Procedure to measure cell death mediated by transfected NMDA receptors using the CytoTox 96® Non-Radioactive Cytotoxicity Assay.**



**Figure 2. HEK 293 cells transfected with NMDA receptor wild-type and NMDA R1(N598Q) mutant subunits alone or in combination and subsequently cultured in the presence or absence of the competitive antagonist, AP5. Media were collected and cell mortality was determined using the CytoTox 96® Non-Radioactive Cytotoxicity Assay as outlined in Figure 1. These results show that co-expression of the R1/R2A receptor subtype yields cell death, in contrast to the putative subtype, R1/R2C. Further, the expression of the mutant R1(N598Q)/R2A, a point mutation known to reduce the calcium permeability of the channel, results in reduced cell mortality post-transfection. Thus, these results establish a novel means to screen for important domains with NMDA receptor subtypes. Three separate transfections were performed for each NMDA receptor subunit combination.**

## Summary

Using the CytoTox 96® Non-Radioactive Cytotoxicity Assay, we have developed a novel means to study the assembly and properties of functional NMDA receptors, the co-association of the different subunits and the effect of point mutations on the ability of the cloned NMDA receptors to flux calcium.

## References

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## Ordering Information

Product	Size	Cat. #
CytoTox 96® Non-Radioactive Cytotoxicity Assay	10 96 well plates	G1780

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