



Cell Technology, Inc

aCella –TOX

Bioluminescence Non Radioactive Cytotoxicity Assay

Key Benefits

- Safe - Non Radioactive Enzyme release assay
- Versatile - Assay can be run in serum supplemented media
- Homogenous - One-step, no wash assay. Assay can be run in same plate as samples
- FAST - Results in 3-5 minutes
- Highly Sensitive - can detect less than 500 cells/well in 10% FCS
- Adaptable for High Throughput format
- Non-destructive assay allows monitoring of additional parameters.
- Perfect for ADCC and CMC assays

Introduction

Cell Technology introduces **aCella-TOX**, a new and highly sensitive assay using its recently patented Coupled Luminescent technology for the detection of cytotoxicity. This assay is designed to quantitatively measure the release of Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) from mammalian cell lines or bacterial cells ^(1,2,3,4).

Other enzyme release assays, for example the Lactate Dehydrogenase (LDH) release assay ^(5,6,7,8), suffer from low sensitivity as a result of interference by serum or phenol red present in the media. **aCella-TOX** can work in both these media formulations and allowing overnight assays while retaining sensitivity.

Assay Principle

GAPDH is an important enzyme in the glycolysis and gluconeogenesis pathways. This homotetrameric enzyme catalyzes the oxidative phosphorylation of D-glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate in the presence of cofactor and inorganic phosphate.

The release of G3PDH is coupled with the enzyme 3-Phosphoglyceric Phosphokinase (PGK) to produce ATP. ATP is detected via the luciferase, luciferin Bioluminescence methodology. Further, ATP is detected with **aCella-TOX** as a homogenous assay that can measure cytotoxicity or cell viability in the same plate. Additionally, culture supernatants can be removed from the original assay plate and assayed in a different plate, allowing kinetic assays to be set up. A further feature is that the assay is non-destructive which allows monitoring of additional parameters such as gene expression.

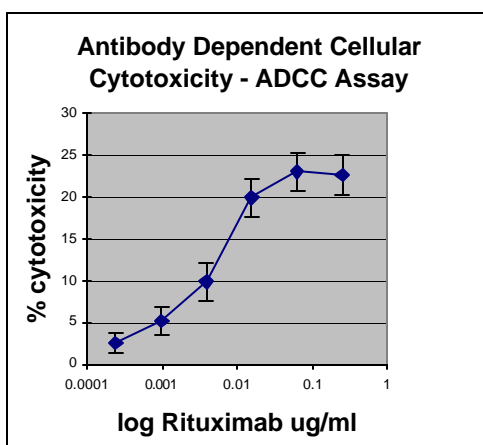


Fig. 1 ADCC: 10,000 Ramos cells/well were incubated with serially diluted Rituximab antibody (humanized anti CD20) for 15 minutes prior to the addition of freshly isolated effector cells. The ADCC assay was further incubated for a total of 4 hours and % cytotoxicity detected using the aCella Tox assay. T:E ratio 1:16.

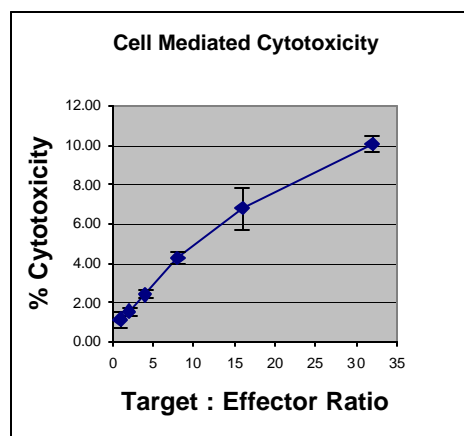


Fig. 2 CMC: 10,000 Jurkat cells/well were mixed with freshly isolated Peripheral Blood Lymphocytes at various Target : Effector cell ratios. The CMC assay was incubated for 4 hours and % cytotoxicity detected using the aCella Tox assay

Kit Contents

1. Lyophilized Enzyme Assay Reagent.....Part# 6001
2. Enzyme Assay Diluent A.....Part# 3008
3. Vial of Detection Reagent.....Part# 6002
4. Detection Assay Diluent B.....Part# 3009
5. Glyceraldehyde 3-Phosphate (G3P).....Part# 6003
6. Lytic Agent.....Part# 3035

Ordering Information

Catalog #	Size	Price (US\$)
CLATOX 100-3	500	595
CLATOX 100-4	1000	1145

References:

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Citations

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3. Corey, M. J. and Kinders, R. J. (2005) "Coupled Luminescent Methods in Drug Discovery: 3-Min Assays for Cytotoxicity and Phosphatase Activity" Drug Discovery Handbook, Ed. Shayne Cox Gad, published by John Wiley & Sons, Inc., pp. 689-731

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