EnzyLight™ Cytotoxicity Assay Kit (ECTX-100)
Bioluminescent Assay for Cytotoxicity

DESCRIPTION

Adenosine 5'-triphosphate (ATP) is the chemical energy for cellular metabolism and is often referred to as “energy currency" of the cell. ATP is produced only in living cells during photosynthesis and cellular respiration and consumed in cellular processes including biosynthetic reactions, motility and cell division. It is a key indicator of cellular activity and has been utilized as a measure of cell viability and cytotoxicity in research and drug discovery.

BioAssay Systems' EnzyLight™ Cytotoxicity Assay Kit provides a rapid method to measure intracellular ATP, cell viability and cytotoxicity. The single working reagent lyses cells to release ATP, which, in the presence of *luciferase*, immediately reacts with the Substrate *D*-luciferin to produce light. The light intensity is a direct measure of intracellular ATP concentration and hence number of living cells.

$$\text{ATP} + \textit{D}\text{-luciferin} + O_2 \xrightarrow{\textit{Luciferase}} \text{oxyluciferin} + \text{AMP} + PP_i + CO_2 + \text{light}$$

This non-radioactive, homogeneous cell-based assay can be conveniently performed in microplates. The reagent is compatible with all culture media and liquid handling systems for high-throughput screening applications in 96-well and 384-well plates.

KEY FEATURES

Safe. Non-radioactive assay (cf. ^{3}H-thymidine incorporation assay).
Sensitive and accurate. As low as 50 cells can be quantified.
Homogeneous and convenient. "Mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.
Robust and amenable to HTS: Z' factors of 0.6 to 0.7 are routinely observed in 96-well and 384-well plates. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

Cell proliferation: effects of cytokines, growth factor, nutrients.
Cytotoxicity and apoptosis: evaluation of toxic compounds, anti-cancer antibodies, toxins, environmental pollutants etc.
Drug discovery: high-throughput screening for anticancer drugs.

KIT CONTENTS

Assay Buffer:	**10 mL**
Substrate:	**120 µL**
ATP Enzyme:	**120 µL**

Storage conditions: The kit is shipped on ice. Store all reagents at -20°C. Shelf life: 6 months after receipt.
Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

ASSAY PROCEDURE IN 96-WELL PLATES

1. *Cell Culture*. Plate cells at 100 µL/well in white opaque tissue culture plates. If desired, add 5 µL test compounds and controls dissolved in PBS or culture medium per well. Rock plate lightly to mix and incubate for desired period of time (e.g. overnight).

2. *Assay*. Bring all components to room temperature. Keep thawed ATP Enzyme on ice or 4°C. For each test well, mix 95 µL Assay Buffer with 1 µL Substrate and 1 µL ATP Enzyme. Add 90 µL Reconstituted Reagent to each test well and mix by tapping the plate. Incubate for 2 minutes at room temperature.

Read luminescence on a luminometer. For most luminometers (Berthold Luminometer, LJL Analyst, Top Count, MicroBeta Counters, CLIPR and LeadSeeker), integration time of 0.1 to 5 sec is appropriate.

ASSAY PROCEDURE IN 384-WELL PLATES

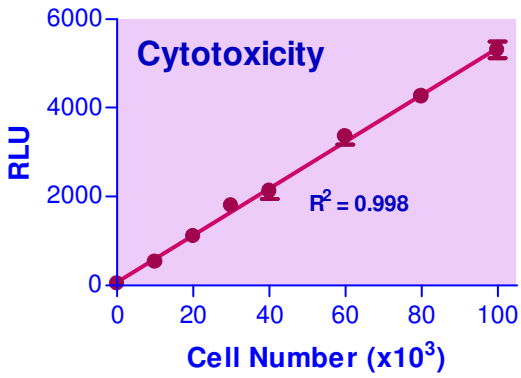
1. *Cell Culture*. Plate cells at 25 µL/well in white opaque tissue culture plates. If desired, add 5 µL test compounds and controls dissolved in PBS or culture medium per well. Rock plate lightly to mix and incubate for desired period of time (e.g. overnight).

2. *Assay*. Bring all components to room temperature. Keep thawed ATP enzyme on ice or 4°C. For each test well, mix 30 µL Assay Buffer with 0.3 µL Substrate and 0.3 µL ATP Enzyme. Add 25 µL Reconstituted Reagent to each well and mix by tapping the plate. Incubate for 2 minutes at room temperature.

Read luminescence on a luminometer. For most luminometers (Berthold Luminometer, LJL Analyst, Top Count, MicroBeta Counters, CLIPR and LeadSeeker), integration time of 0.1 to 5 sec is appropriate.

GENERAL CONSIDERATIONS

Signal stability. After adding the Reconstituted Reagent, the luminescence signal is stable for about 15 min and decreases slow thereafter. Reading is best performed within 30 min.



Linearity of Luminescence to Cell Number in 96-well Plate Assay

LITERATURE

[1]. Li W. et al (2006). Human primary renal cells as a model for toxicity assessment of chemo-therapeutic drugs. *Toxicol In Vitro*. 20(5):669-76.

[2]. Zhelev Z, et al (2004). Phenothiazines suppress proliferation and induce apoptosis in cultured leukemic cells without any influence on the viability of normal lymphocytes. Phenothiazines and leukemia. *Cancer Chemother Pharmacol*. 53(3):267-75.

[3]. Ingram PR, et al (2004). A comparison of the effects of ocular preservatives on mammalian and microbial ATP and glutathione levels. *Free Radic Res*. 38(7):739-50.