

# EZMTT™ Cell Proliferation Assay

Cell Proliferation Assay

Cat. # CBA410

pack size: 1000 Assays

FOR RESEARCH USE ONLY.  
NOT FOR USE IN DIAGNOSTIC PROCEDURES.  
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Store at -20°C



Data Sheet

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## Background

EZMTT™ cell proliferation assay is a novel monosulfonated tetrazolium salt, 2-(3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2H-tetrazol-3-ium-5-yl) benzenesulfonate sodium salt. The EZMTT™ reagents detect oxidoreductase enzymes, indicators of cellular metabolic activity, with high sensitivity and reproducibility. Upon reaction, EZMTT™ is converted to a yellowish formazan that does not require a solubilization step and can be quantified by absorbance at 450 nm. EZMTT™ assay can be used to measure cell proliferation, cytotoxicity or cytostatic activity (shift from proliferation to quiescence) of potential medicinal or toxic agents.

### Features and Benefits of EZMTT™

- Can be premixed with any media and then directly added to the cells
- Is very stable and has little cytotoxicity. A longer incubation from days to weeks is possible
- Reduction of EZMTT™ produces a water-soluble yellow colored formazan dye, which does not require solubilization step
- More sensitive than the traditional MTT assays, and can continuously track the cell growth easily by absorbance at 450 nm or by color changes in both mammalian and bacterial cells
- Much less reactive with other antioxidants such as beta-Mercaptoethanol (BME)

## Storage

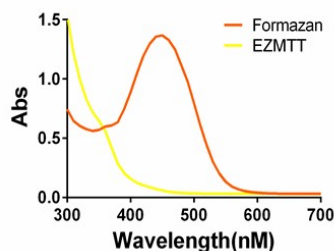
Store EZMTT™ cell proliferation assay solution below -20°C, and protect from light. Can withstand multiple freeze & thaw cycles.

## Spectral Properties

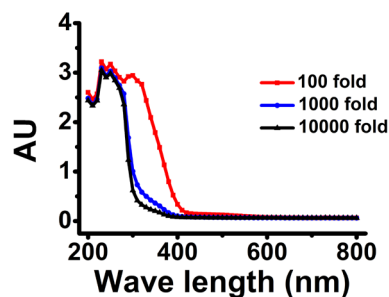
Absorbance: 450 nm

## Quality Control

Purity: ≥ 98% confirmed by TLC or NMR. Quality confirmed by NADH titration of the product, dose response to *E. coli* and A549 cell culture.



**Figure 1:** Absorbance spectra of EZMTT™ and its metabolic product, formazan.



**Figure 2.** UV spectrum of EZMTT™ that has been diluted for 100-fold, 1000-fold and 10000-fold in DMSO. Total volume 180µL in 96 well plate. Flexstaton 3 was used measure absorbance. The product has maximal absorption at 320 nm, as expected.

## Protocol

### Assay Protocol

1. Detach Hela cells and seed different cell number (0, 2500, 5000, 10000, 20000, 30000, 40000, 50000) in each well of 96-well plate with 10% FBS DMEM in triplicates.
2. Incubate at 37°C, 5% CO<sub>2</sub> for 3-4 hours or overnight. Then change the culture medium with 100 µl of 10% FBS DMEM (optional).
3. Thaw and mix 200X EZMTT™ cell proliferation assay solution with culture media (20-fold dilution).
4. Add 10 µl of the 10X EZMTT™ solution to each well of the plate (final 200-fold dilution).
5. Incubate the plate for 1-4 hours in the incubator. Measure the absorbance at 450 nm using a microplate reader.

## References

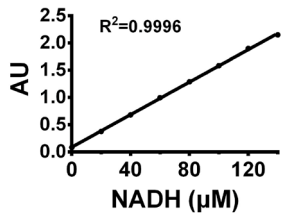
1. Zhang W, et al. Mono-sulfonated tetrazolium salt-based NAD(P)H detection reagents suitable for dehydrogenase and real-time cell viability assays. *Anal Biochem.* 509, 15 Sept 2016, 33-40.
2. Hu Q, et al. Detection of "Hidden" Antimicrobial Drug Resistance. *ACS Infectious Diseases.* 2019, 5, 1252-1263
3. Ruan J, et al. Kidney-Type Glutaminase Inhibitor Hexylselen Selectively Kills Cancer Cells via a Three-Pronged Mechanism. *ACS Pharmacology & Translational Science,* 2019, 2: 18-30.
4. Rui J, et al. The EZMTT cell proliferation assay provides precise measurement for drug combinations and better correlation between in vitro and in vivo efficacy. 2020. *Bioorganic & Medicinal Chemistry Letters,* 30, 127134-127239.

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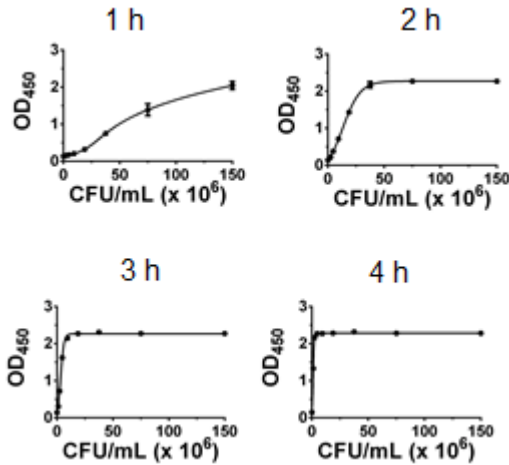
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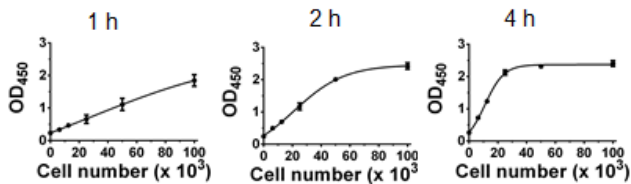
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**Figure 3.** NADH (0-140 µM; final) titration of 1X EZMTT™ solution is a linear curve for absorption at 450 nm, as expected. A total volume of 200µL in 96-well plate was used and detected by Flexstation 3.



**Figure 4.** Dose response of *E. coli* ATCC25922 (0-150\*10<sup>6</sup> cells/96-well) to 1X EZMTT™ cell proliferation assay for 1-4 hours.



**Figure 5.** Dose response of A549 cells (0-1\*10<sup>5</sup> cells/96well) to 1X EZMTT™ cell proliferation assay for 1-4 hours.

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