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 -20ºC

Data Sheet

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.

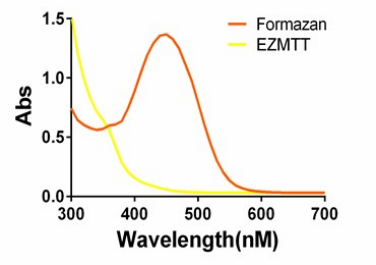

Figure 1: Absorbance spectra of EZMTT™ and its metabolic product, formazan.

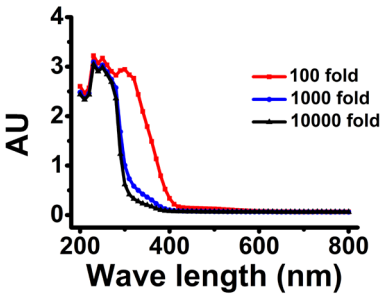

Figure 2. UV spectrum of EZMTT ™ that has been diluted for 100-fold, 1000-fold and 10000-fold in DMSO. Total volume 180µL in 96 well plate. Flexstaton 3 was used measure absorbance. The product has maximal absorption at 320 nm, as expected.

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 37ºC, 5% CO_2 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. 2016. 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.

Rev.1.0/2020-06-09/CBA410/MD

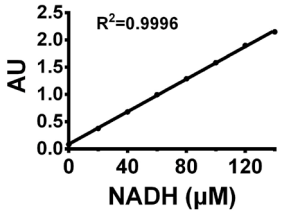

Figure 3. NADH (0-140 µM; final) titration of 1X EZMTT™ solution is a linear curve for absorption at 450 nm, as expected. A total volume of 200µL in 96-well plate was used and detected by Flexstation 3.

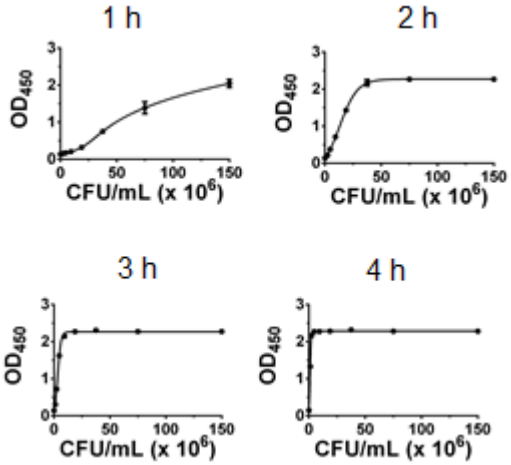

Figure 4. Dose response of *E. coli* ATCC25922 (0-150*10^6 cells/96-well) to 1X EZMTT™ cell proliferation assay for 1-4 hours.

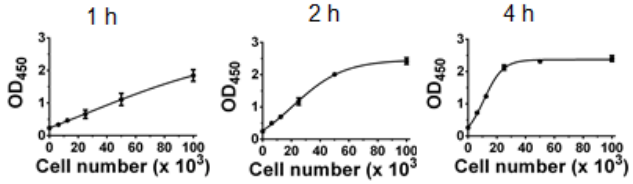

Figure 5. Dose response of A549 cells (0-1*10^5 cells/96well) to 1X EZMTT™ cell proliferation assay for 1-4 hours.