# EZMTT™ High Sensitivity Cell Viability Assay

**Cell Viability Assay** 

# Cat. # CBA411

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

## pack size: 1000 Assays

Store at -20°C



# **Data Sheet**

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#### Background

EZMTT<sup>TM</sup> high sensitivity cell viability 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 (EZMTT). The EZMTT<sup>TM</sup> reagents provide highly sensitive and reproducible detection for oxidoreductase enzymes, indicators of cellular metabolic activity. Upon reacting with metabolic enzymes, EZMTT <sup>TM</sup> is converted to a yellowish formazan that does not require a solubilization step and can be measure by absorbance at 450 nm. EZMTT <sup>TM</sup> assay can be used for end-point cell viability, cytotoxicity (loss of viable cells), or cytostatic activity (shift from proliferation to quiescence) of potential medicinal or toxic agents.

Features and Benefits of EZMTT<sup>™</sup> high sensitivity cell viability assay:

- · Can be premixed with any media and then added to the cells
- EZMTT<sup>™</sup> is more sensitive than the traditional MTT assays and can be used to detect metabolic activity of low-density cell cultures
- Reduction of EZMTT<sup>™</sup> produces a water-soluble formazan dye, which does not require a solubilization step
- Can be used for end-point cell viability assay of mammalian as well as bacterial cells
- Stable, much less reactive with other antioxidants such as beta-Mercaptoethanol (BME)

#### Storage

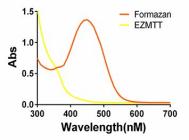
Store EZMTT<sup>™</sup> high sensitivity cell viability assay solution at -20°C, and protect from light.

## **Spectral Properties**

Absorbance: 450 nm

## **Quality Control**

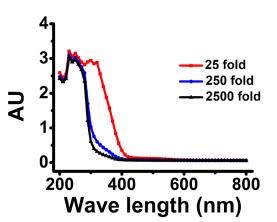
Purity:  $\ge$  98% confirmed by TLC or NMR. Quality confirmed by NADH titration of the product, dose response to E. coli and A549 cell culture.



#### Protocol

#### **Assay Protocol**

- Detach Hela cells and seed different cell number (0, 1000, 2000, 5000, 10000) in each well of 96-well plate with 100 μl of 10% FBS DMEM in triplicates.
- 2. Incubate at 37°C, 5%  $CO_2$  for 3-4 hours or overnight. Then change the culture medium with 100 µl of 10% FBS DMEM (optional).
- Thaw and Mix 50X EZMTT <sup>™</sup> cell viability assay solution with culture media (5-fold dilutions).
  Add 10 µl of the 10X EZMTT <sup>™</sup> solution to each well of the plate
- Add 10 µl of the 10X EZMTT<sup>™</sup> solution to each well of the plate (final 50-fold dilution).
- 5. Incubate the plate for 1-4 hours in the incubator.
- Measure the absorbance at 450 nm using a microplate reader.



**Figure 2.** UV spectrum of EZMTT <sup>TM</sup> that has been diluted for 25-fold (2X final), 250-fold (0.2X final), 2500-fold (0.02X final) in DMSO. Total volume 180  $\mu$ L in 96 well plate. Detected by Flexstaton 3. The product has maximal absorption at 320 nm, as expected.

#### References

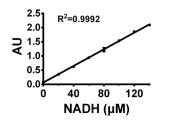
- Zhang W, et al. Mono-sulfonated tetrazolium salt-based NAD(P)H detection reagents suitable for dehydrogenase and real-time cell viability assays. 2016. Anal Biochem. 509, 15 Sept 2016, 33-40.
- Yu Y, et al. Ebselen: Mechanisms of Glutamate Dehydrogenase and Glutaminase Enzyme Inhibition. ACS Chem Bio. 2017, 12, 12, 3003-3011.

Figure 1: Absorbance spectra of  $\mathsf{EZMTT}^{\mathsf{TM}}$  and its metabolic product, formazan.

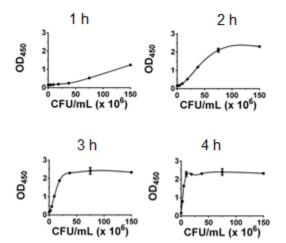
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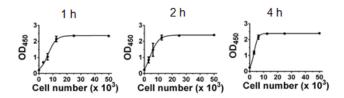
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**Figure 3.** NADH (0-140  $\mu$ M, final) titration of EZMTT <sup>TM</sup> solution is a linear curve for absorption at 450 nm, as expected. A total volume of 200  $\mu$ L in 96 well plate was used and detected by Flexstaton 3.



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



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

EZMTT<sup>™</sup> is a trademark of JNF Bioscience

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