EZ-CYTOX

Cell Viability, Proliferation & Cytotoxicity Assay Kit

Cat. No. EZ-500
EZ-1000
EZ-3000
EZ-5000
EZ-BULK150

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

DoGenBio Co.,Ltd.

▪ Product Description

EZ-CYTOX is a product that measures the amount of living cells using WST and can be used for Cell Viability, Proliferation & Cytotoxicity Assay, etc. WST is a High Sensitive Water Soluble Tetrazolium Salt that is highly soluble in water and reacts with dehydrogenase in live cells to produce orange-colored water-soluble formazan. Dehydrogenase, which reacts with WST, is an enzyme present in the mitochondrial electron transport chain of metabolically active cells and is effective only in live cells. Therefore, the production of formazan has a linear correlation with the number of viable cells, which can be determined by measuring the absorbance (450 nm).

EZ-CYTOX is a sterilized One Bottle Solution that can be used without preparation before use. There is no need to dissolve formazan or remove the culture medium, so you can easily conduct experiments on suspension cells.

▪ Kit Contents

Catalog No.	Assay	Qty.
EZ-500	500 tests	5 ㎖ x 1 bottle
EZ-1000	1000 tests	5 ㎖ x 2 bottles
EZ-3000	3000 tests	5 ㎖ x 6 bottles
EZ-5000	5000 tests	25 ㎖ x 2 bottles
EZ-BULK150	10000 tests	25 ㎖ x 4 bottles

▪ Storage and Stability

- Store at 0~4℃ and can be used without change in activity for 1 year from the date of manufacture.
- It can be used for more than 2 years when stored at -20℃. But, repeated freezing and thawing may reduce activity and increase background.

▪ Blank

Mix 100 ㎕ of the culture medium used in the experiment (without cells) with 10 ㎕ of EZ-Cytox and use it as a blank.

Please set a blank because absorbance can be measured due to the type of medium, incubation time, and light exposure.
(Usually, it is measured around 0.3 for 1 hour reaction.)

▪ Optimization of Cell Concentration (option)

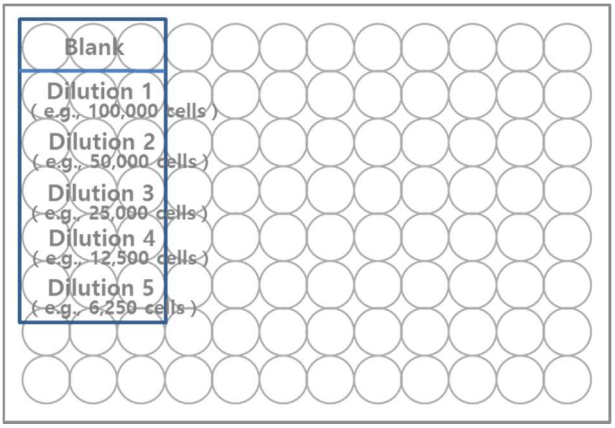
Since the number of cells to be used in the experiment and the reaction time after addition of EZ-Cytox vary depending on the type of cell and culture conditions, it is recommended to determine it through a preliminary experiment. This is a necessary process even when experimenting with other solutions such as MTT and MTS.

1. Collect the cells in culture and prepare a cell suspension to obtain an appropriate cell number.

   * Determine the appropriate cell number by referring to reference literature and existing experiments.
   ex) Viability, Proliferation assays = 0.1 – 5 x 10^4 cells/well
   Cytotoxicity assays = 2 x 10^4 – 5 x 10^5 cells/well

2. Serial dilute the prepared cell suspension. (3~5 set)

3. Dispense 100㎕ of the prepared cell suspension per well into a 96-well plate and pre-incubate in an incubator. (e.g., at 37℃, 5% CO_2 for 2~24 hrs)



* The cell numbers in the figure are examples. Decide based on cell type and literature.

4. Incubate for an appropriate time according to the experimental conditions.

   * The incubation time is based on the time for adding and reacting the test substance during this experiment. Because this is a preliminary experiment to set the experimental conditions, no experimental substances are added. (e.g., 6, 12, 24, 48 hours)

5. Add 10 ㎕ of EZ-Cytox to each well and culture in the incubator.

6. Determine the appropriate cell number and incubation time with EZ-Cytox by checking the absorbance with a plate reader from 30 minutes to a maximum of 4 hours. (O.D. 450 nm)

   * Gently shake for about 1 minute before measuring absorbance. Usually, the absorbance is measured every 30 minutes and measure the absorbance, then return the plate to the incubator.

   * The recommended incution time is 1 hour.

▪ Protocol – Cell Proliferation & Cytotoxicity Assay

1. Prepare a cell suspension, dispense 100㎕ per well into a 96-well plate, and pre-incubate in a CO2 incubator. (e.g., at 37℃, 5% CO_2 for 2~24hrs)
2. Add 10 ㎕ of test substances (e.g. toxicant) prepared at various concentrations to each well.
3. Incubate for an appropriate time according to the experimental conditions. (e.g. 6, 12, 24, 48 hrs)
4. Add 10 ㎕ of EZ-Cytox to each well.
   * When using yellow or dark-colored experimental substances (plant extracts, etc.) or reducing agents, washing is required. Please refer to Note 4.
5. Incubate for about 0.5 to 4 hours.
6. Gently shake for about 1 minute before measuring absorbance.
7. Measure absorbance at 450 nm using a plate reader. (Reference wavelength 600~650nm)

▪ Note

1. Since the incubation time after addition of EZ-CYTOX varies depending on the type or number of cells, for optimal experimental results before performing the main experiment, it is recommended to determine the number of cells to be used in the experiment and the optimal incabation time through a preliminary experiment.

2. If there are air bubbles when measuring absorbance, accuracy may decrease. It is recommended to remove air bubbles before measuring absorbance.

3. If the test substance is a reducing agent, it may react with the WST in EZ-CYTOX to form formazan. If you plan to use a reducing agent, it is best to check it by measuring absorbance before experiment.

4. If washing is necessary before adding EZ-Cytox, wash 2 to 3 times using PBS. When using the suction system, cells may be sucked out and affect the results, so if possible, attach a multi-channel pipette to the wall of the well and wash them out. After washing, add 100 ㎕ of culture medium to each well. Proceed with the experiment starting with protocol number 4.

   * option : You may add 10% EZ-Cytox to the culture medium and mix it in advance.
     Ex. : Mix 10 ㎖ of media + 1 ㎖ of EZ-Cytox, dispense 100 ㎕ into each well, and proceed from protocol number 5.

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▪ Calculation of viability

$$\text{Viability (\%)} = \frac{\text{Exp. - Blank}}{\text{Control - Blank}} \times 100$$

Blank : absorbance of a well *medium and EZ-Cytox* , without cell
Control : absorbance of a well with *cell and EZ-Cytox*, without test solution
EXP. : absorbance of a well with *cell, test solution and EZ-Cytox*

▪ Q & A

Q1: What is the difference between other products that use MTT, MTS, and XTT and EZ-CYTOX that uses WST?

Although the basic measuring principle is the same, the solubility of formazan produced by each tetrazolium salt in water is different. In the case of MTT, since it forms formazan in the form of a crystal that is insoluble in water, a cumbersome process of dissolving it using an organic solvent such as DMSO or a surfactant is required. MTS and XTT are variants of MTT and form water-soluble formazan, but It has lower solubility and stability compared to WST. Therefore, EZ-CYTOX, which uses WST, has the advantage of higher sensitivity and a wider measurement range than other products.

In addition, by upgrading the sensitivity of WST with our technology, it is possible to measure with sensitivity equal to or better than that of other products that use WST.

Q2: What wavelength range can be used when measuring absorbance after adding EZ-CYTOX?

We recommend using 450 nm.
If there is no 450 nm filter, measurements can be made at wavelengths between 420-480 nm.

Q3: Is it possible to use wells of other sizes other than 96 well?

It can be used not only for 96 wells, but also for all micro well plates such as 6, 24, 48, and 384 wells, as well as dishes of various sizes. In this case, add EZ-CYTOX at 1/10 of the total volume.
(ex : In the case of 200 ㎕ of cell in media + 100 ㎕ of reaction material, the total volume is 300 ㎕, so just add 1/10 of 30 ㎕ of EZ-CYTOX to the well.)

Q4: When observed under a microscope, many cells appear to be dead, but the results of an experiment with EZ-CYTOX show that the viability is high. Why is that so?

a. In this case, most of the cells observed to be dead are actually damaged cells, which still have NADH-dehydrogenase activity and react with EZ-CYTOX. If these damaged cells need to be counted as dead for the purpose of the experiment, they must be removed by washing out, etc. and then measured again.
b. It may occur when EZ-Cytox is reduced by an experimental substance.
In this case, please refer to note 4 and proceed with the experiment after washing.

Q5: I would like to do another test using the cells tested with EZ-CYTOX. Is it possible?

EZ-CYTOX has little cell toxicity and does not affect cells, so using EZ-CYTOX after the assay is completed, other experiments such as DNA or RNA extraction and western blot are possible.

Q6: Why measure absorbance at reference wavelength?

Measure other factors that affect experimental results.
(Curvature of the well plate, turbidity of the medium, etc.)
Normally, the absorbance at the reference wavelength is measured to be around 0.05.

▪ Reference

1. Zhegang Huang, et al., Nature Communications. 2011, 2,459,
2. Cheol Am Hong, et al., J. Am. Chem. Soc., 2011, 133 (35), pp 13914–13917
3. Gwang Sig Yu, et al., Bioconjugate Chem., 2011, 22 (6), pp 1046–1055
4. Jae-Hyuk Jang, et al., J. Am. Chem. Soc., 2011, 133 (18), pp 6865–6867
5. Sang Un Lee, et al., J. Nat. Prod., 2011, 74 (5), pp 1284–1287

▪ Related Product

Product	Catalog No.	Assay
EZ-Cytox Plus*	EZ-3000P	3000 tests
LDH Assay Kit**	DG-LDH500	500 tests
	DG-LDH1000	1000 tests

* This product has improved sensitivity compared to EZ-Cytox and is used when reaction time is long due to slow metabolic activity or when high-sensitivity experiments must be performed with a small number of cells.

** A kit that can measure cytotoxicity with high sensitivity by measuring the LDH enzyme released from dead cells. Since the supernatant is used, two types of data can be obtained in one experiment when used in parallel with the EZ-Cytox experiment.