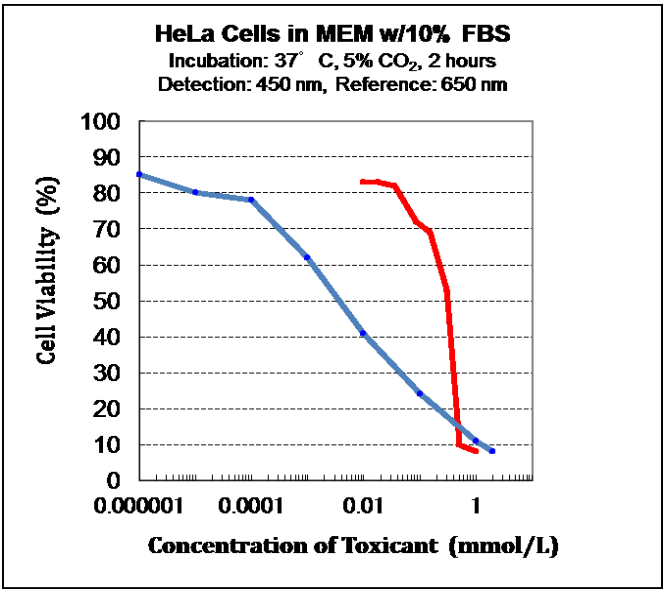
Northwest
Life Science Specialties, LLC

Premier Products for Superior Life Science Research

NWLSS™ Cytotoxicity/CellViability Assay

Product NWK-CTOX01

For Research Use Only



Simple assay kit for determining levels of cytotoxicity and/or cell proliferation as a percentage of viable cells remaining.

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Introduction:

Cytotoxicity, cell proliferation or viability assays are common requirements in many laboratories. Among these, historical methods such as Tritiated [^{3}H] thymidine and thymidine analog (BrdU) require the use of radioactive substances and/or are tedious and time consuming. Additionally, both of these methods require special equipment or detection methods to perform. In contrast, methods based on tetrazolium salts including MTT, XTT, MTS and water soluble WST-1 and WST-8 are quick, efficient and easy to perform in standard microplates without the need of specialized equipment such as scintillation counters or antibody detection apparatus.

The NWLSS™ Cytotoxicity/Cell Viability Assay is based on the least toxic of the aforementioned salts, WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium, monosodium salt].

Advantages of the WST-8 based method:

- Single reagent with no pre-mixing of reagents required.

- Superior stability with 6 Months @ 25°C, 12 Months @ 4°C & 24+ Months @ -20°C

- Non Radioactive: good correlation with tritiated [^{3}H] thymidine & thymidine analog (BrdU) assays.

- Phenol red containing culture media can be used with cell viability assays.

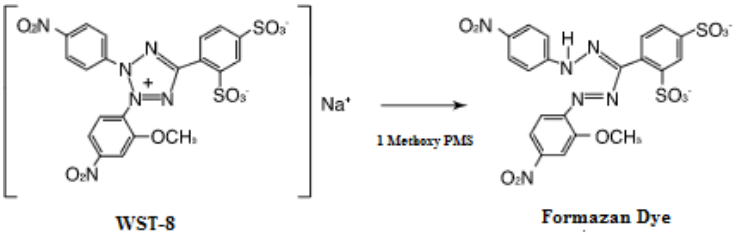
- Lowest toxicity of all tetrazolium salt compounds. Therefore, additional tests are possible after testing is performed with this reagent.

- More sensitive at detecting dehydrogenase activity than other tetrazolium salts such as MTT, XTT, MTS or WST-1.

Intended Use:

The NWLSS™ Cytotoxicity/Cell Viability Assay product is designed for use as a tool for researchers to easily determine the impact of various substances on the viability of cultured cell lines.

Test Principle:
Cellular dehydrogenases react with WST-8 in the presence of an electron mediator. Reduction of WST-8 in this fashion yields an orange colored, water soluble formazan product with a peak absorbance at 450 nm.



WST-8 Formazan Dye

The amount of dehydrogenase mediated color development is proportional to the number of living cells and easily measured using a standard micro-plate reader with 450 nm capabilities (typical ELISA absorbance after STOP).

General Specifications:
Format: Colorimetric

Number of tests: 500 tests (@ 10 µl / 100 µl medium)

Specificity: Specific for cellular dehydrogenase activity

Kit Contents:
Test Reagent containing 1-Methoxy PMS electron mediator....1 X 5.0 mL

Required Materials Not Provided:
CO_2 incubator
96-well plate
Pipettes for delivering 10 and 100 - 200 µl.

Required Instrumentation:
Microplate reader with 450 nm capability.
Note: The λ_{max} for WST-8 Formazan dye is 450 nm. However, color can be measured anywhere between 430 nm and 490 nm.

Warnings, Limitations, Precautions:

1. This assay is based on the dehydrogenase activity of viable cells. Therefore, any condition or chemical that can affect dehydrogenase activity may cause a discrepancy between true viable cells and viable cells determined using this assay.

2. WST-8 can react with other reducing agents to generate WST-8 formazan. Please check the background O.D. if reducing agents are used in cytotoxicity assays or cell proliferation assays.

3. Bubbles can interfere with the O.D. reading.

4. If necessary, membrane filtration may be used to for sterilization of the reagent.

5. Requisite incubation times will vary by the type and number of cells per well. For instance, leukocytes typically yield weaker coloration. For leuckocytes and/or larger numbers of cells (~10^5cells/well) longer incubation times of up to 4 hours may be necessary.

6. For samples with high turbidity, it is recommended that absorbance at 600 nm or higher be recorded and subtracted from the regular 450 nm measurement.

Storage Instructions:

Recommended storage is 4 °C when reagent is expected to be used frequently. Do not use components beyond the expiration date printed on the label.

Assay Preparation

Plate Reader Setup Recommendations

Set wavelength to record at 450 nm.

Reagent Preparation:

WST-8 Reagent is supplied ready to use.

Sample Handling/Preparation:

Recommended number of cells per well for testing in a 96 well plate:

Adhesive cells:

1000+ cells are recommended per well with 100 µl medium.

Leukocytes:

2500+ cells per well are recommended per well with 100 µl medium.

Maximum per 96 well plate:

25000 is the recommended max number of cells per 100 uL medium.

Sample Handling/Preparation (continued):
Determining the number of cells per well for testing in other plate sizes:

For 24 and 6 well plates, requisite cell numbers can be calculated according to the 96 well "per 100 µL medium guidelines" (e.g. 1X cell number per 100 µL medium).

Assay Protocol:
Baseline Cell Number Determination for Standard 96 Well Procedure
1. Add 100 µl cell suspension to each well as necessary for testing.

2. Pre-incubate the cells in a humidified incubator (e.g., at 37°C, 5% CO_2).

3. Add 10 µl of **Test Reagent** to each well.

4. Incubate the plate for 1 - 4 hours (depending on sample type) in the incubator.

5. Measure the absorbance at 450 nm using a microplate reader.

Cell Proliferation and/or Cytotoxicity Assay
1. Add 100 µl of cell suspension to each well as necessary for testing.

2. Harvest of adherent cells can disrupt normal growth activities. For this reason, it is necessary to pre-incubate adherent cells for 24 hours in a humidified incubator at 37°C, 5% CO_2 in order to return them to a normal growth cycle. This step can be omitted for non-adherent cells

3. Add 10 µl of substance to be tested to the plate.

4. Incubate the plate for an appropriate length of time (e.g., 6, 12, 24 or 48 hours) in the incubator.

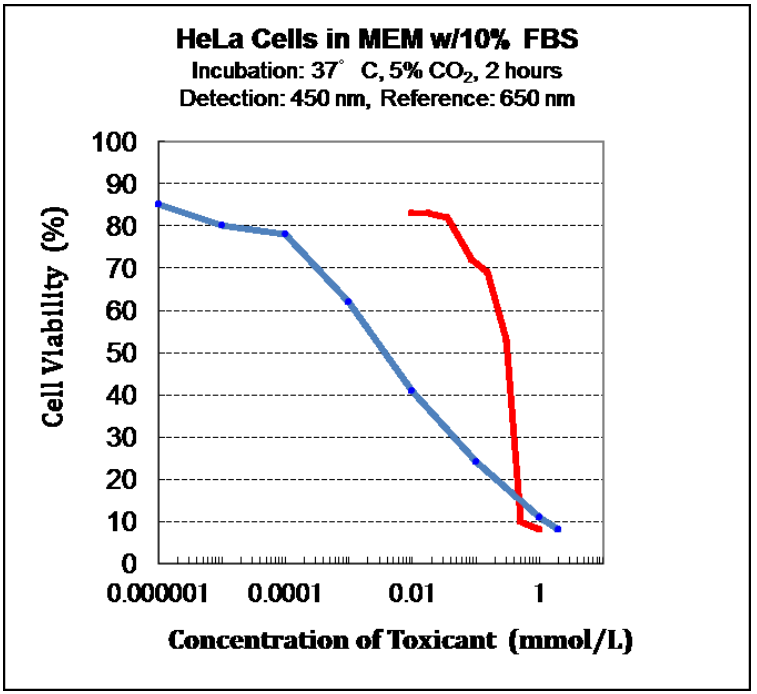
5. Add 10 µl of **Test Reagent** to each well.

6. Incubate the plate for 1 - 4 hours in the incubator.

7. Measure the absorbance at 450 nm using a microplate reader.

Note: The absorbance can be read up to 24 hours later by adding 10 µl of 1% w/v SDS or 0.1 M HCl to each well, covering the plate and storing it protected from light at room temperature (25°C).

Data Analysis:
Sample Chemical Toxicity Data:

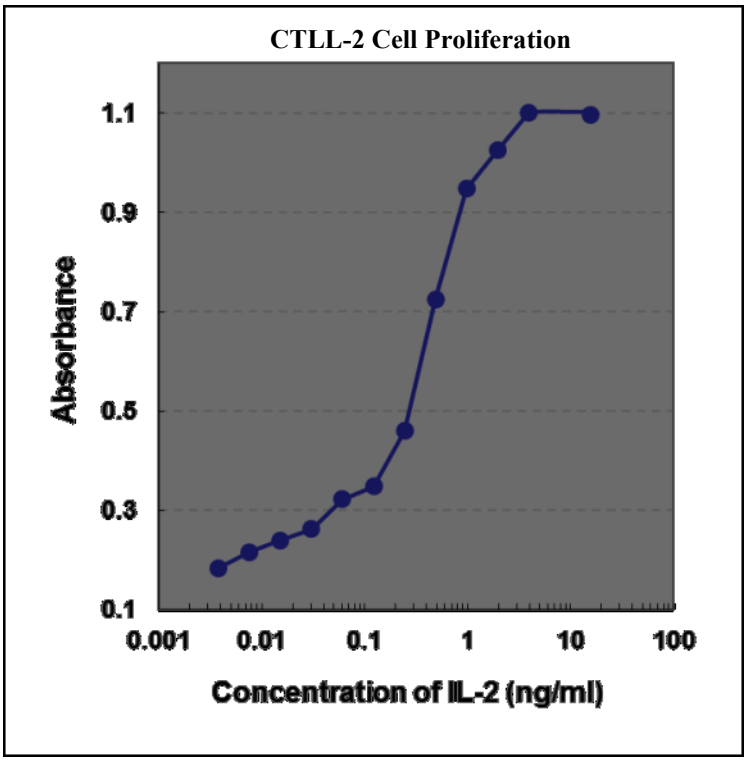


As can be seen in the figure above, the Test Reagent is effective in highlighting the dose response in terms of declining viability with increasing chemical dosage.

Cell survival rate may be calculated by comparing the surviving cell absorbance with that of the baseline "normal" cell absorbance. It is recommended that an absorbance blank consisting of Test Reagent plus medium be subtracted from each sample.

$$Survival\ Rate = \frac{Post\ Intervention - Blank}{Pre\ Intervention - Blank}\ X\ 100$$

Data Analysis (continued):
Sample Proliferation Data



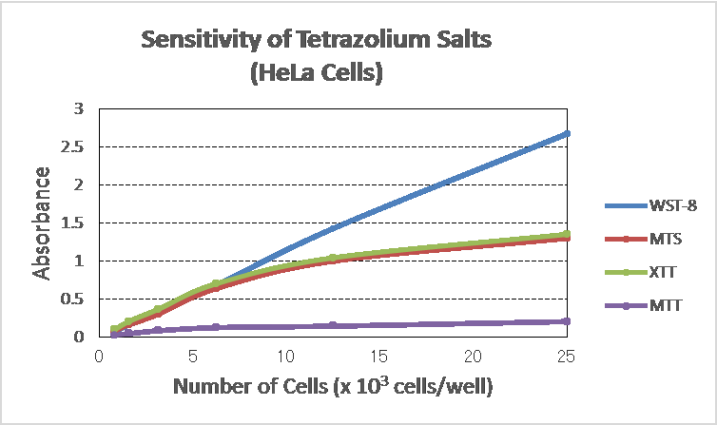
Used as a proliferation assay, the Test Reagent clearly illustrates the dose dependent response of cells to intervention, in this case IL-2 administration.

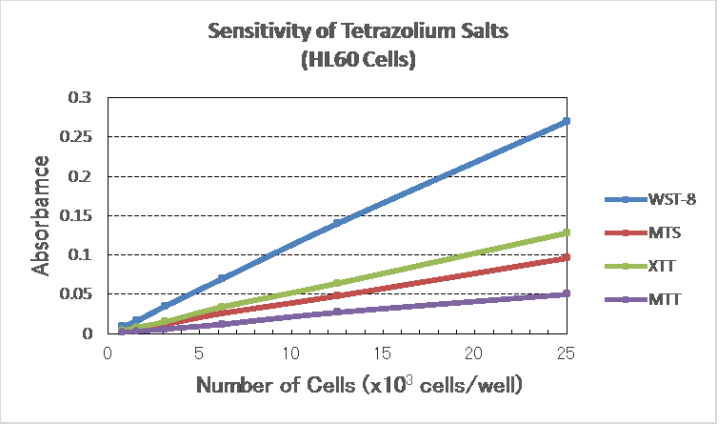
Cell proliferation rate may be calculated by comparing the surviving cell absorbance with that of the baseline "normal" cell absorbance. It is recommended that an absorbance blank consisting of Test Reagent plus medium be subtracted from each sample.

$$Proliferation\,Rate = \frac{Post\ Intervention - Blank}{Pre\ Intervention - Blank}\ X\ 100$$

Performance Details:

Sensitivity of WST-8 based Test Reagent in relation to other commonly used compounds.





Stability

25 °C = 6 months

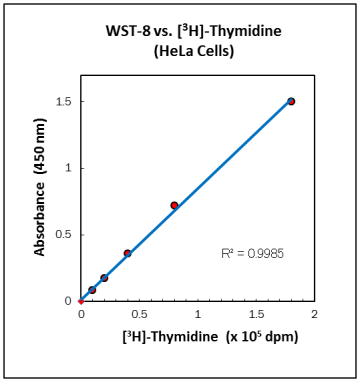
4 °C = 1 year if protected from light

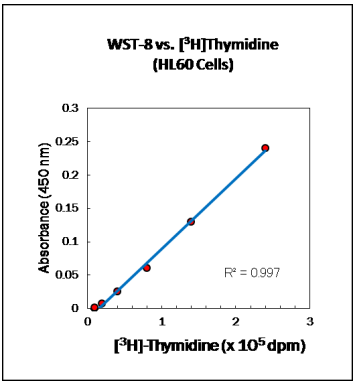
-20 °C = 2 years*

*Note: Repeated freeze /thaw conditions result in increased background.

Performance Details (continued):

Comparison of WST-8 absorbance and cytotoxicity as measured using tritiated [^{3}H] thymidine assay.





The WST-8 method shows excellent correlation with traditional [^{3}H] thymidine methods and can easily be substituted for this type of assay. However, since these are two totally different techniques, users are cautioned not to directly compare data across the 2 assay types.

References:

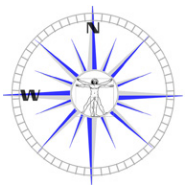
1. M. Ishiyama, Y. Miyazono, K. Sasamoto, Y. Ohkura and K. Ueno, Talanta, **1997**, 44, 1299.

2. H. Tominaga, M. Ishiyama, F. Ohseto, K. Sasamoto, T. Hamamoto, K. Suzuki and M. Watanabe, Anal. Commun., **1999**, 36, 47.

Statement of Limited Warranty:

Northwest Life Science Specialties, LLC (NWLSS) makes no guarantee of any kind, expressed or implied, that extends beyond the description of the material in this kit, except that they will meet our specifications at the time of delivery. Customer's remedy and NWLSS' sole liability is limited to, at NWLSS' option, refund of the purchase price, or the replacement of material not meeting our specification. By acceptance of our product, customer assumes all liability and will indemnify and hold NWLSS harmless for the consequence of this product's use or misuse by the customer, its employees, or others. Refund or replacement is conditioned of customer notifying NWLSS within twenty-one (21) days of the receipt of product. Failure to give notice within 21 days shall constitute a waiver by the customer of all claims hereunder with respect to said product.

End-User Notes:



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