WST-1 Cell Proliferation Assay Kit

Catalog No. 10008883

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GENERAL INFORMATION

Materials Supplied

No.	Item	96 Well Quantity/Size	480 Well Quantity/Size	Storage
1	WST-1 reagent, powder	1 vial	1 vial	-20°C
2	Electron Mediator solution	1 vial/1 ml	1 vial/5 ml	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

WARNING: This product is not intended or approved for use in humans or veterinary animals. Reliance on this product for analyte measurements in a therapeutic setting is hazardous and may result in illness or injury.

Precautions

Please read these instructions carefully before beginning this assay. For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

E-Mail: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Adjustable pipettes and a repeat pipettor
2. A 96-well plate for culturing cells
3. A 96-well plate reader capable of absorbance at 450 nm

INTRODUCTION

Background

Cell proliferation is controlled by growth factors that bind to cell surface receptors which connect to signaling molecules. These molecules activate transcription factors which bind to DNA to modulate the production of proteins, resulting in cell division. Dysfunction of any step in this regulatory cascade causes abnormal cell proliferation, an underlying cause of many human pathological conditions, most notably cancer and aging.[1] Defining mechanisms responsible for alterations in cell cycle progression is crucial to understanding many human diseases, most notably cancer.

Cell proliferation assays have been widely used to assess cell cycle regulatory factors such as growth factors, cytokines, mitogens, and drugs.[2] These assays have evolved from classical [^{3}H]-thymidine incorporation, to 5'-bromo-2'-deoxy-uridine (BrdU) incorporation, to WST-1, WST-8, MTT, or XTT methods. In comparison to the traditional radioactive assay or the time consuming BrdU assay, WST-1, WST-8, MTT, and XTT have the advantage of being easy to perform in a microtiter plate without washing steps. These assays can be completed within three to four hours.

About This Assay

Cayman's WST-1 cell proliferation assay kit provides an easy to use tool for studying induction and inhibition of cell proliferation in any *in vitro* model. The assay is based on the enzymatic cleavage of the tetrazolium salt WST-1 to formazan by cellular mitochondrial dehydrogenases present in viable cells. This kit will also allow investigators to screen drug candidates involved in regulation of cell cycle.

PRE-ASSAY PREPARATION

Reagent Preparation

Reagents

1. WST-1 Reagent (vial #1)
2. Electron Mediator Solution (vial #2)

Procedure

Immediately before use, thaw the electron mediator solution (vial #2) and use it to reconstitute the entire vial of WST-1 reagent (vial #1). Mix well. If the entire vial of reconstituted WST-1 reagent will not be used in a single experiment, we recommend that you aliquot and store it at -20°C. When stored at -20°C, the reconstituted WST-1 reagent will be stable for several months. Avoid repeated freeze/thaw cycles.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical experimental plate will include wells without cells, wells with cells treated with experimental compounds and wells of untreated cells. We recommend that each treatment be performed in triplicate and that you record the contents of each well on the template sheet provided (see page 11).

Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps to maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (i.e., slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Procedure

1. Seed cells in a 96-well plate at a density of 10^4-10^5 cells/well in 100 µl of culture medium with or without compounds to be tested. Culture the cells in a CO_2 incubator at 37°C for 24-48 hours.
2. Add 10 µl of the reconstituted WST-1 mixture to each well using a repeating pipettor.
3. Mix gently for one minute on an orbital shaker.
4. Incubate the cells for two hours (adherent culture) to four hours (suspension culture) at 37°C in a CO_2 incubator.
5. Before reading the plate, it is important to mix gently on an orbital shaker for one minute to ensure homogeneous distribution of color.
5. Measure the absorbance of each sample using a microplate reader at a wavelength of 450 nm.

ANALYSIS

Sample Data

An example of typical data obtained with this assay is shown in the figure below. Your data will vary depending on the cell line and culture conditions used. It is important that the initial plating of the cells is sparse enough to allow for linear cell growth during the experiment. This is particularly important with adherent cells, which may undergo contact inhibition as they near confluence. The optimal number of cells to plate can be determined by performing a cell titration experiment, as shown in Figure 1, below.

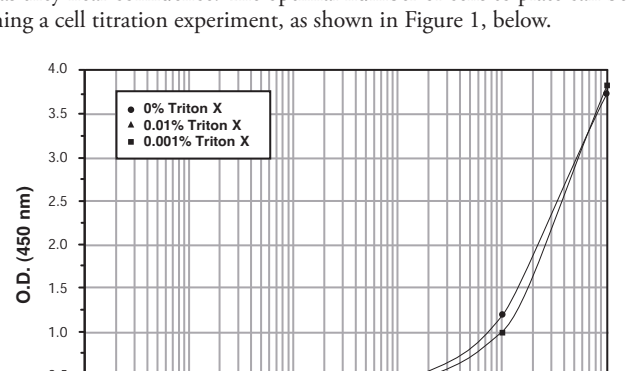


Figure 1: Effect of cell number on absorbance at 450 nm. CHO cells were seeded in a 96-well plate in 100 µl of culture medium at the density indicated in the figure and incubated overnight at 37°C in a CO_2 incubator. The following day, the cells were treated with 0.001% Triton X-100, 0.01% Triton X-100 or vehicle, and gently shaken on an orbital shaker for 10 minutes at room temperature. WST-1 was added to each well, mixed gently for one minute, then the plate was incubated at 37°C in a CO_2 incubator. After two hours of incubation, the absorbance was measured at 450 nm.

Assay Range

The assay can detect from 10^3-10^5 cells, depending on cell type.

RESOURCES

References

1. Šulic, S., Panic, L., Đikic, I., *et al.* Deregulation of cell growth and malignant transformation. *Croat. Med. J.* **46(4)**, 622-638 (2005).
2. Francoeur, A.-M. and Assalian, A. Microcat: A novel cell proliferation and cytotoxicity assay based on WST-1. *Biochemica* **3**, 19-25 (1996).

Related Products

Caspase-3 Fluorescence Assay Kit - Cat No. 10009135
Cholesterol Cell-Based Detection Assay Kit - Cat. No. 10009779
D-*myo*-Inositol-1,3,4,5,6-pentaphosphate (sodium salt) - Cat. No. 10007784
E-64c - Cat. No. 10007964
JC-1 Mitochondrial Membrane Potential Assay Kit - Cat. No. 10009172
LDH Cytotoxicity Assay Kit - Cat. No. 10008882
LDH Diaphorase - Cat. No. 10009318
LDH NAD+ (100X) - Cat. No. 10009319
MTT Cell Proliferation Assay Kit - Cat. No. 10009365
N-Ac-Asp-Glu-Val-Asp-CHO - Cat. No. 10017
N-Ac-DEVD-N'-MC-R110 - Cat. No. 10007014
N-Ac-Tyr-Val-Ala-Asp-CHO - Cat. No. 10016
9Z,11E,13E-Octadecatrienoic Acid - Cat. No. 10008349
9Z,11E,13E-Octadecatrienoic Acid ethyl ester - Cat. No. 10008350
9Z,11E,13E-Octadecatrienoic Acid methyl ester - Cat. No. 10008333
PD 98059 - Cat. No. 10006726
Stearic Acid ethyl ester - Cat. No. 10008196
WST-8 Cell Proliferation Assay Kit - Cat. No. 10010199

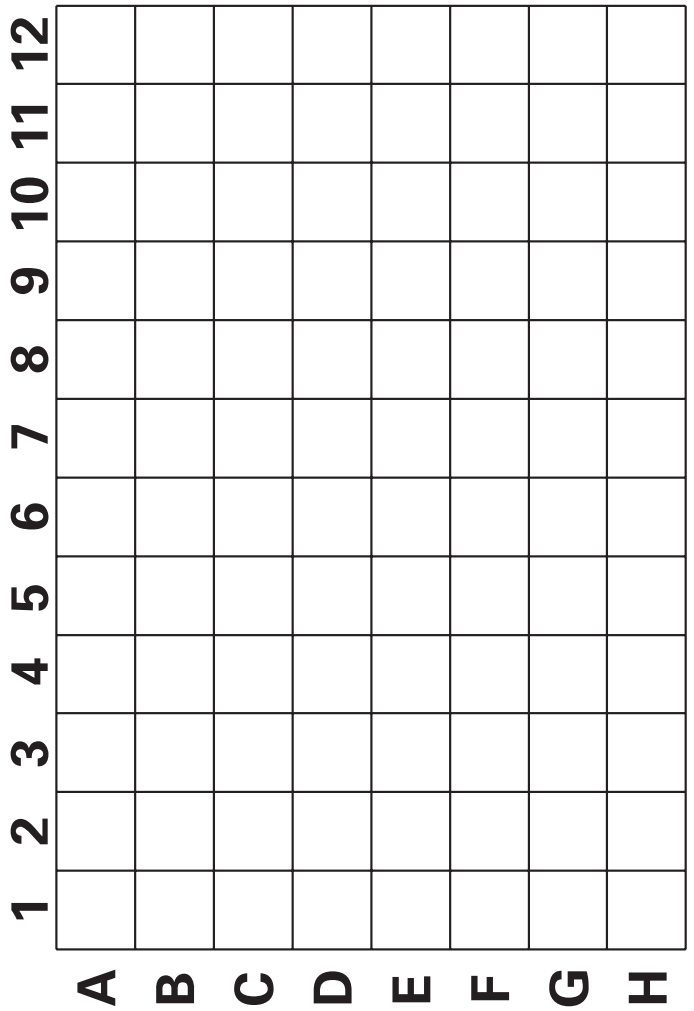
Warranty and Limitation of Remedy

Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material **will meet our specifications at the time of delivery**. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's exclusive remedy and Cayman's sole liability hereunder shall be limited to a **refund** of the purchase price, or at Cayman's option, the **replacement**, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



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