

WST-1 Cell Proliferation Assay Kit

Item No. 10008883



Customer Service 800.364.9897 * **Technical Support** 888.526.5351

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 Well Quantity/Size	480 Well Quantity/Size	4,800 Well Quantity/Size	Storage
10008976	WST-1 Reagent (powder)	1 vial/1 ea	1 vial/5 ea	10 vials/5 ea	-20°C
10008977	Electron Mediator Solution	1 vial/1.2 ml	1 vial/6 ml	1 vial/60 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) 971-3335. We cannot accept any returns without prior authorization.



WARNING: This product is for laboratory research use only; not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

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 repeating pipettor.
2. A 96-well plate for culturing cells.
3. A 96-well plate reader capable of measuring 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 [³H]-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 (powder) (Item No. 10008976)
2. Electron Mediator Solution (Item No. 10008977)

Procedure

Immediately before use, thaw the Electron Mediator Solution and use it to reconstitute the WST-1 Reagent (powder) as described below.

For 96-well or 480-well sizes, reconstitute the entire vial of WST-1 Reagent (powder) (Item No. 10008976) with the vial of Electron Mediator Solution (Item No. 10008977). 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.

For the 4,800-well size, thaw the entire vial of Electron Mediator Solution (Item No. 10008977) and make 6 ml aliquots. To each vial of the WST-1 Reagent (powder) (Item No. 10008976), add one 6 ml aliquot of the Electron Mediator Solution. Mix well. The WST-1 Reagent and the Electron Mediator Solution should be stable for up to one year when stored separately at -20°C. Once reconstituted, the mixture will be stable for several months when stored at -20°C. 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

- Use different tips to pipette each reagent.
- 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 μ l of culture medium with or without compounds to be tested. Culture the cells in a CO₂ incubator at 37°C for 24-48 hours.
2. Add 10 μ l 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 CO₂ 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.

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.

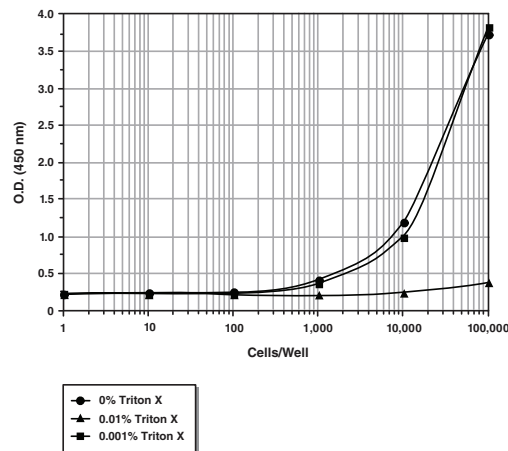


Figure 1: Effect of cell number on absorbance at 450 nm. CHO cells were seeded in a 96-well plate in 100 μ l of culture medium at the density indicated in the figure and incubated overnight at 37°C in a CO₂ incubator. The following day, the cells were treated with 0.001% Triton X-100, 0.01% Triton X-100 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 CO₂ incubator. After two hours of incubation, the absorbance was measured at 450 nm.

Assay Range

The assay can detect from 10³-10⁵ cells, depending on cell type.

References

- Šulic, S., Panic, L., Đikic, I., *et al.* Deregulation of cell growth and malignant transformation. *Croat. Med. J.* **46**(4), 622-638 (2005).
- 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

Apoptotic Blebs Assay Kit - Item No. 10010750
 Caspase-3 Fluorescence Assay Kit - Item No. 10009135
 Cathepsin S Cell-Based Assay Kit - Item No. 600740
 Cholesterol Cell-Based Detection Assay Kit - Item No. 10009779
 Glutathione Cell-Based Detection Kit (Blue Fluorescence) - Item No. 600360
 Glycerol Cell-Based Assay Kit - Item No. 10011725
 Glycolysis Cell-Based Assay Kit - Item No. 600450
 JC-1 Mitochondrial Membrane Potential Assay Kit - Item No. 10009172
 LDH Cytotoxicity Assay Kit - Item No. 10008882
 Lysosome/Cytotoxicity Dual Staining Kit - Item No. 600310
 MTT Cell Proliferation Assay Kit - Item No. 10009365
 Multi-Parameter Apoptosis Assay Kit - Item No. 600330
 NAD⁺/NADH Cell-Based Assay Kit - Item No. 600480
 Neutrophil Elastase Activity Assay Kit - Item No. 600610
 Neutrophil Myeloperoxidase Activity Assay Kit - Item No. 600620
 Oxygen Consumption/Glycolysis Dual Assay Kit - Item No. 601060
 Oxygen Consumption/MitoMembrane Potential Dual Assay Kit - Item No. 600880
 Oxygen Consumption Rate Assay Kit (MitoXpress®-Xtra HS Method) - Item No. 600800
 Perfecta3D® Cell Viability Kit - Item No. 600990
 Perfecta3D® LDH Cytotoxicity Assay Kit - Item No. 601050
 WST-8 Cell Proliferation Assay Kit - Item No. 10010199
 XTT Cell Proliferation Assay Kit - Item No. 10010200

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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NOTES

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