ab65475

WST-8 Assay Kit (Cell Proliferation)

Instructions for Use

For the rapid, sensitive and accurate measurement of Cell Proliferation in cell culture (adherent and suspension)

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Abcam's WST-8 Assay Kit (Cell Proliferation) provides by far the easiest and most sensitive means for quantifying cell proliferation and viability.

The assay is based on the cleavage of the tetrazolium salt to formazan by cellular mitochondrial dehydrogenase. The amount of the dye generated by activity of dehydrogenase is directly proportional to the number of living cells. The formazan dye produced by viable cells can be quantified by multi-well spectrophotometer (microtiter plate reader) by measuring the absorbance of the dye solution at 440 nm.

The assay can be used for measurement of cell proliferation in response to growth factors, cytokines, mitogens, and nutrients, etc. It can also be used for the analysis of cytotoxic compounds like anticancer drugs and many other toxic agents and pharmaceutical compounds. The new method is so simple, just add-and-read, requiring no washing, no harvesting, and no solubilization steps. It is faster, stable, and more sensitive than MTT, XTT, MTS based assays. The assay correlates well with the [3H]-thymidine incorporation assay.

2. Protocol Summary

Culture Cells

⇩

Add WST Solution

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Incubate Cells

⇩

Shake Thoroughly

⇩

Measure Absorbance

3. Components and Storage

A. Kit Components

Item	Quantity (500 assays)	Quantity (2500 assays)
WST-8 Reagent	1 vial	5 vial
Electrocoupling Solution	5 ml	25 ml
Stop Solution IV	5 ml	25 ml

PLEASE NOTE: WST-8 Reagent was previously labelled as WST Reagent I and WST Reagent (Lyophilized). The composition has not changed.

* Store kit at -20°C.

WST-8 REAGENT: Dissolve the vial of lyophilized WST-8 Reagent into 1 mL of Electrocoupling Solution. Further dilute by adding the 1 mL of reconstituted WST to 4 mL of Electrocoupling Solution. Aliquot the solution (1 ml is sufficient for one 96-well plate assay) and store at -20°C.

The WST Solution is stable for 1 year at -20°C and up to 6 months at +4°C. Protect from light. Avoid repeated freeze-thaw. Repeated freeze-thaw may increase background.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Multi-well spectrophotometer (microtiter plate reader)
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Culture cells (0.1-5x10^4/well) in a 96-well microtiter plate in a final volume of 100 µl/well culture medium in the absence or presence of various amounts of the factors tested.

   For toxicity assays, use more cells to start with (e.g., 5x10^4-x10^5 cells/well).

   The optimal cell number used for the assay may vary among cell types. For best results, it is recommended to add various numbers of cells in your initial assay to determine the optimal cell number and the developing time to be used.

2. Incubate cells for 24-96 hours.

3. Add 10 µl per well WST Solution to each well. Be careful not to introduce bubbles to the wells.

4. Incubate the cells for 0.5-4 hours in standard culture conditions.

5. Shake thoroughly for 1 min. Measure the absorbance of the treated and untreated samples using a microtiter plate reader at 420-480 nm according to the filters available for the plate reader. The reference wavelength should be ~650nm.

Notes:

a) Use the same amount of culture medium and WST-8 Reagent in an empty well as a blank position for the microtiter plate reader.

b) The plate can be repeatedly read many times until OD reaches 1.8.

c) The reaction can be stopped by adding 10 μl of the Stop Solution IV into each well, mix well, and the plate can be read within 48 hours. Protect from light and prevent medium evaporation.

d) Phenol Red in culture medium does not significantly interfere with the assay.

e) WST shows very low toxicity and it does not stain the cells. Thus, the same cells can be used for other tests after WST assay.

f) Vortex WST-8 Reagent to dissolve material during reconstitution instructions.

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

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