ab155902
WST-1 Cell Proliferation Reagent (ready to use)

For the measurement of cell proliferation in cultured cells.

View kit datasheet: www.abcam.com/ab155902
(use www.abcam.cn/ab155902 for China, or www.abcam.co.jp/ab155902 for Japan)

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 4a, Last Updated June 5, 2023

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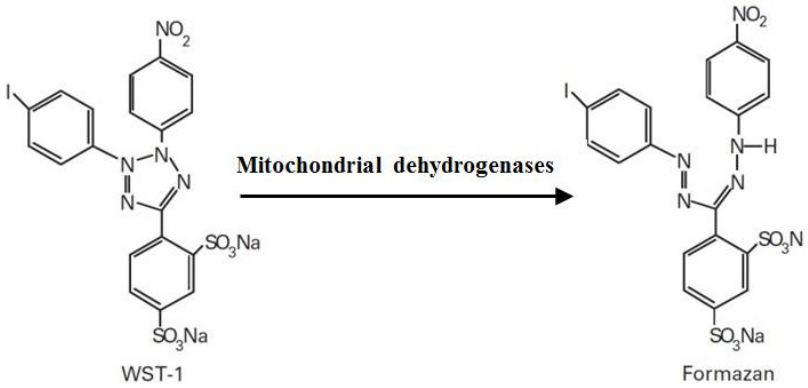
1. Overview

WST-1 Cell Proliferation Reagent (ready to use) (ab155902) provides a simple and accurate method to measure cell proliferation. This method is based on the cleavage of the tetrazolium salt WST-1 to formazan by mitochondrial dehydrogenases.

Increase in the number of viable cells leads to an increase in the activity of mitochondrial dehydrogenases, which in turn results in an increase in the amount of formazan dye produced. The formazan dye produced from WST-1 by viable cells can be quantified by measuring the absorbance of the dye at OD=440 nm.

WST-1 is more sensitive than MTT, XTT or MTS-based assays, and the entire assay can be performed in the sample microtiter plate and does not require additional steps like washing, harvesting or cell solubilization.



Applications:

- Measurement of cell proliferation in response to growth factors, cytokines, mitogens and other stimuli
- Analysis of cytotoxic and/or cytostatic compounds such as anticancer drugs, toxic agents and other pharmaceuticals.
- Assessment of physiological mediators that can inhibit cell growth.

2. Protocol Summary

Grow cells and treat as desired

Add WST-1 Reagent I/WST-1 reagent

Incubate cells 30 min – 4 hours

Measure absorbance at OD420 – 480 nm

Determine change as percentage of control after background subtraction

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition (Before prep)	Storage Condition (After prep)
WST-1 Reagent I/WST-1 Reagent	2500 test (25 mL)	-20°C	-20°C/4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 420 – 480 nm
- MilliQ water or other type of double distilled water (ddH_2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- General cell culture supplies
- Optional: 1% SDS solution (to stop reaction)

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 WST-1 Reagent I/WST-1 Reagent:

Ready to use as supplied. Aliquot reagent so that you have enough volume to perform the desired number of assays (1 mL is enough to test 1 x 96-well plate). Avoid freeze/thaw.

Reagent is stable for 3 weeks at 4°C and 6 months at -20°C.

10. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all controls and samples in duplicate.
- WST-1 Reagent I/WST-1 reagent incubation time depends on the individual cell type and cell concentration used. Therefore, we recommend that you determine the optimal incubation time for the particular experimental setup used.
- Phenol red present in the culture medium does not significantly interfere with the reading.

10.1 Grow 0.1 – 5 x 10^4 cells/well in a 96 well microtiter plate in a final volume of 100 µL culture medium. Grow cells in the absence or presence of compounds of interest.

- Growth assays = 0.1 – 5 x 10^4 cells/well
- Cytotoxicity assays = 5 x 10^4 – 5 x 10^5 cells/well

10.2 Incubate cells for 24 – 96 hours.

10.3 Add 10 µL WST-1 Reagent I/WST-1 reagent to each well.

- Blank control wells: 100 µL culture medium + 10 µL WST-1

Δ Note: increase or decrease amount of WST-1 reagent according to the volume of culture medium used.

10.4 Incubate cells for 0.5 – 4 hours in standard culture conditions.

10.5 Shake plate for 1 minute on a shaker to mix contents.

10.6 Measure absorbance of control (untreated) and treated samples using a microplate reader at OD = 420 – 480 nm, depending on the filters available. The reference wavelength should be 650 nm.

10.7 Optional: assay can be stopped by adding 10 µL of 1% SDS into each well.

11. Data Analysis

CELL PROLIFERATION ASSAYS

- Average the duplicate reading for each sample.
- Subtract the culture medium background from your assay reading. This is the corrected absorbance.
- Amount of absorbance is proportional to cell number.

Δ Note: for cell counting, a standard curve can be established with known cell number and fixed incubation times with the assay reagent.

CELL CYTOTOXICITY ASSAYS

- Average the duplicate reading for each sample.
- Subtract the culture medium background from your assay readings. This is the corrected absorbance.
- Calculate percentage cytotoxicity with the following equation, using corrected absorbance:

$$\%\ Cytotoxicity = \frac{(100\ x\ (Control - Sample))}{Control}$$

12. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold reagent	Warm reagent to assay temperature
	Plate read at incorrect wavelength	Check equipment and filter settings of instrument
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter settings of instrument

13. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)