For life science research only.
Not for use in diagnostic procedures.

Cell Proliferation Reagent WST-1

Colorimetric assay (WST-1 based) for the nonradioactive quantification of cell proliferation, cell viability, and cytotoxicity

Cat. No. 11 644 807 001 25 ml for 2500 tests
Cat. No. 05 015 944 001 8 ml for 800 tests

Version 16
Content version: February 2011

Store at −15 to −25°C
⚠ Store protected from light!

1. What this Product Does

Contents

The Cell Proliferation Reagent WST-1 is a clear, slightly red, ready-to-use solution, containing WST-1 and an electron coupling reagent, diluted in phosphate buffered saline, filtered through 0.2 µm pore size membrane.

Number of Reactions

Cat. No. 11 644 807 001	for 2500 tests
Cat. No. 05 015 944 001	for 800 tests

Storage and Stability

The unopened reagent is stable when stored at −15 to −25°C, protected from light, through the expiration date printed on the label.

If precipitates or turbidity are observed upon thawing, warm up the solution to 37°C for 2 – 10 min and agitate to dissolve the precipitates. Centrifugation is not recommended because the working concentration would decrease. After being dissolved, the WST-1 reagent can be used without any limitations. Please store as follows:

- Once thawed, store at +2 to +8°C, protected from light, for up to four weeks. However please note that the solution may become viscous. If so, warm up the solution to 37°C for 2 – 10 min as described above.
- For longer storage it is recommended to store in aliquots at −15 to −25°C.

⚠ The kit is shipped on dry ice.

Additional Equipment and Reagents Required

Additional reagents and equipment required to perform reactions include:

- Incubator (37°C)
- Centrifuge
- Microplate (ELISA) reader with a filter for a wavelength between 420 – 480 nm (if a reference wavelength is to be subtracted, a filter above 600 nm is recommended).
- Microscope
- Hemacytometer
- Multichannel pipettor (10, 50, 100 µl)
- Sterile pipette tips
- 96-well microplates

For the Cell proliferation assay:

- Culture medium, *e.g.,* RPMI 1640 containing 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 1 mM Na-pyruvate, 1× non-essential amino acids, and 50 µM 2-mercaptoethanol.
- Optionally, add Penicillin/Streptomycin* or Gentamicin
- Human IL-2 (10,000 U/ml; 5 µg/ml), sterile filtered

For the Cytotoxicity assay (TNF-α):

- Culture medium, *e.g.,* RPMI 1640 containing 10% heat-inactivated FCS, 2 mM L-glutamine, and actinomycin C1 (actinomycin D), 1 µg/ml.
- Optionally, add Penicillin/Streptomycin or Gentamicin.
- Human TNF-α (10 µg/ml), sterile filtered.

Application

The Cell Proliferation Reagent WST-1 is designed to be used for the non-radioactive, spectrophotometric quantification of cell proliferation, growth, viability, and chemosensitivity in cell populations using the 96-well-plate format. It can be used *e.g.,* for:

- The measurement of cell proliferation in response to growth factors, cytokines, mitogens and nutrients (see figure 1).
- The assessment of growth inhibitory antibodies and physiological mediators (see figure 2).
- Analysis of cytotoxic and cytostatic compounds, such as anti-cancer drugs and other pharmaceutical compounds.

2. How to Use this Product

2.1 Before You Begin

Working concentration

It is recommended to add 10 µl/well Cell Proliferation Reagent WST-1 to the cells already cultured in 100µl/well (1:10 final dilution).

Using the 100 µl/well cell culture volume, one vial will be sufficient to perform 2500 tests (25 microplates).

⚠ If the cells are cultured in 200 µl/well, add 20 µl/well Cell Proliferation Reagent WST-1.

Determination of optimal incubation periods

The appropriate incubation time after the addition of the Cell Proliferation Reagent WST-1 depends on the individual experimental setup (*e.g.,* cell type and cell concentration used). Therefore, it is recommended to measure the absorption repeatedly at different points in time after the addition of the Cell Proliferation Reagent WST-1 (*e.g.,* 0.5, 1, 2, and 4 h) in a preliminary experiment. This allows you to determine the optimal incubation period for the particular experimental setup used (see figure 6).

Incubation requirements for high sensitivity

If high sensitivity is required, incubate the cells in the presence of Cell Proliferation Reagent WST-1 for longer periods of time (Figure 6: half maximum absorbance after incubation with Cell Proliferation Reagent WST-1 for 0.5 h with 2×10^4 cells/well; for 4 h with 0.7×10^4 cells/well).

Initial incubation

If for the initial incubation of the cells an increased volume of culture medium is required, increase the amount of Cell Proliferation Reagent WST-1 correspondingly (*e.g.,* add 20 µl/well Cell Proliferation Reagent WST-1 if cells are cultured in 200 µl/well culture medium).

Control (blank)

Add the same volume of culture medium and Cell Proliferation Reagent WST-1 as used in the experiment into one well (*e.g.,* 100 µl culture medium plus 10 µl Cell Proliferation Reagent WST-1). Use this background control (absorbance of culture medium plus WST-1 in the absence of cells) as a blank position for the ELISA reader.

Background absorbance

Slight spontaneous absorbance occurs if Cell Proliferation Reagent WST-1 is added to culture medium in the absence of cells. This background absorbance depends on the culture medium, the incubation time and exposure to light. Typical background absorbance after 2 h is between 0.1 – 0.2 absorbance units.

2.2 Procedure

The incubation period and cell density of the culture depends on the particular experimental conditions and on the cell line used.

⚠ For most experimental setups, a cell concentration between 0.1 and 5×10^4/well and an incubation time of 24 to 96 h is appropriate

❶	Culture cells in microplates (tissue culture grade, 96 wells, flat bottom) in a final volume of 100 µl/well culture medium in a humidified atmosphere (*e.g.*, 37°C, 5% CO_2).
❷	Add 10 µl/well Cell Proliferation Reagent WST-1.
❸	Incubate the cells for 0.5 to 4 h in a humidified atmosphere (*e.g.,* 37°C, 5% CO_2).
❹	Shake thoroughly for 1 min on a shaker.
❺	Measure the absorbance of the samples against a background control as blank using a microplate (ELISA) reader at 420 – 480 nm. The reference wavelength should be more than 600 nm.

2.3 Cell proliferation assay

Determination of the activity of human interleukin-2 (IL-2) on the mouse T cell line CTLL-2 (see figure 1).

❶	Seed CTLL-2 cells at a concentration of 4×10^3 cells/well in 100 µl culture medium containing various amounts of IL-2 (final concentration *e.g.,* 0.005–25 ng/ml) into microplates (tissue culture grade, 96 wells, flat bottom)
❷	Incubate cells for 48 h at 37°C and 5% CO_2.
❸	Add 10 µl/well Cell Proliferation Reagent WST-1 and incubate for 4 h at 37°C and 5% CO_2.
❹	Shake thoroughly for 1 min on a shaker.
❺	Measure the absorbance of the samples against a background control as blank using a microplate (ELISA) reader. The wavelength for measuring the absorbance of the formazan product is between 420 – 480 nm (max. absorption at about 440 nm) according to the filters available for the ELISA reader (see figure 4). The reference wavelength should be more than 600 nm.

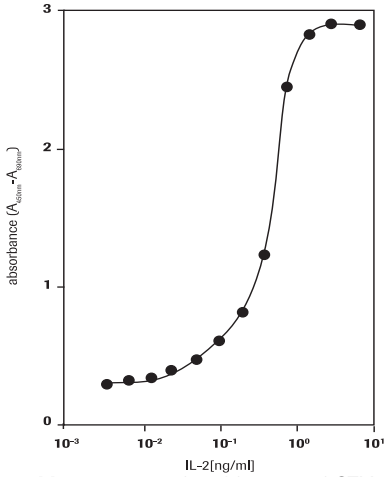

Fig. 1: Measurement of proliferation of CTLL-2 cells in response to human IL-2.

2.4 Cytotoxicity assay (TNF-α)

Procedure for determination of the cytotoxic effect of human tumor necrosis factor-α (TNF-α) on the mouse fibrosarcoma cell line WEHI-164 (see figure 2).

❶	Culture cells in microplates (tissue culture grade, 96 wells, flat bottom) in a final volume of 100 µl/well culture medium in a humidified atmosphere (*e.g.,* 37°C, 5% CO_2).
❷	Seed cells at a concentration of 5×10^4 cells/well in 100 µl culture medium containing actinomycin C1 (1 µg/ml) and various amounts of TNF-α (final concentration *e.g.,* 0.001–0.5 ng/ml) into microplates (tissue culture grade, 96 wells, flat bottom).
❸	Incubate cell cultures for 24 h at 37°C and 5% CO_2.
❹	Add 10 µl Cell Proliferation Reagent WST-1 and incubate for 4 h at 37°C and 5% CO_2.
❺	Shake thoroughly for 1 min on a shaker.
❻	Measure the absorbance of the samples against a background control as blank using a microplate (ELISA) reader. The wavelength for measuring the absorbance of the formazan product is between 420 – 480 nm (max. absorption at about 440 nm) according to the filters available for the ELISA reader (see figure 2). The reference wavelength should be more than 600 nm.

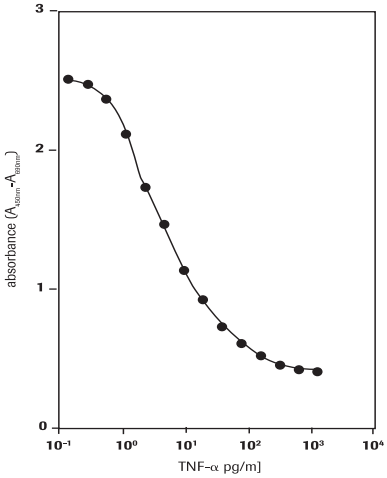

Fig. 2: Determination of the cytotoxic activity of human TNF-α on WEHI- 164 cells.

3. Additional Information on this Product

Product Description

The measurement of cell proliferation and cell viability has become a key technology in the life sciences. The need for sensitive, reliable, fast and easy methods has led to the development of several standard assays (1).

These include the determination of DNA synthesis by measuring the amount of radioactive labeled nucleosides like [^{3}H]-thymidine incorporated in nucleic acid.

Alternatively, the incorporation of 5-bromo-2'-deoxyuridine (BrdU)* in place of thymidine is used to monitor DNA synthesis and cell proliferation in immunohisto- and immunocytochemistry, in cell ELISA and flow cytometry analysis (kits and reagents for these applications are available from Roche Applied Science).

Proliferation assays have become available for analyzing the number of viable cells by the cleavage of tetrazolium salts added to the culture medium. This technique requires neither washing nor harvesting of cells and the complete assay from the onset of the microculture to data analysis by ELISA reader is performed in the same microplate. Together with on-line computer processing consisting of data collection, calculation and report generation, the microtiter tetrazolium assay allows rapid, convenient and automated handling of high number of samples and is thus a viable alternative to the above mentioned methods.

Assay principle

① The tetrazolium salts are cleaved to formazan by cellular enzymes (10). An expansion in the number of viable cells results in an increase in the overall activity of mitochondrial dehydrogenases in the sample. This augmentation in enzyme activity leads to an increase in the amount of formazan dye formed, which directly correlates to the number of metabolically active cells in the culture.

② Quantification of the formazan dye produced by metabolically active cells by a scanning multiwell spectrophotometer (ELISA reader).
The absorbance of the dye solution is measured at appropriate wavelengths.

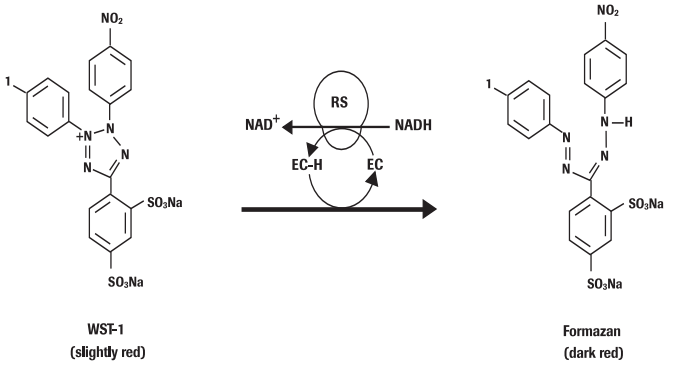

Fig. 3: Cleavage of the tetrazolium salt WST-1 (4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) to formazan. (EC = electron coupling reagent RS = mitochondrial succinate-tetrazolium-reductase system)

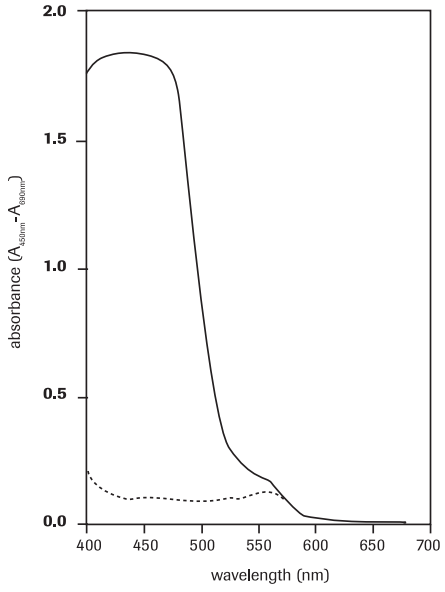

Fig. 4: Absorbance spectra of Cell Proliferation Reagent WST-1 (……) and the reaction product (formazan) (---) after cleavage by mitochondrial dehydrogenase activity. The Cell Proliferation Reagent WST-1 was diluted 1:10 in a cell suspension in RPMI1640 containing 10% FCS

Advantages of WST-1 compared to other cell proliferation agents

The new Cell Proliferation Reagent WST-1 (11–19) (see fig. 5 and 6) has several advantages compared to the above mentioned compounds:

- In contrast to MTT which is cleaved to water-insoluble formazan crystal and therefore has to be solubilized after cleavage, WST-1 yields water-soluble cleavage products like XTT and MTS which can be measured without an additional solubilization step.
- In contrast to XTT and MTS, WST-1 is more stable. Therefore, WST-1 can be used as a ready-to-use solution and can be stored at +2 to+8°C for several weeks without significant degradation.
- WST-1 has a wider linear range and shows accelerated color development compared to XTT (see fig. 5 and fig. 6).

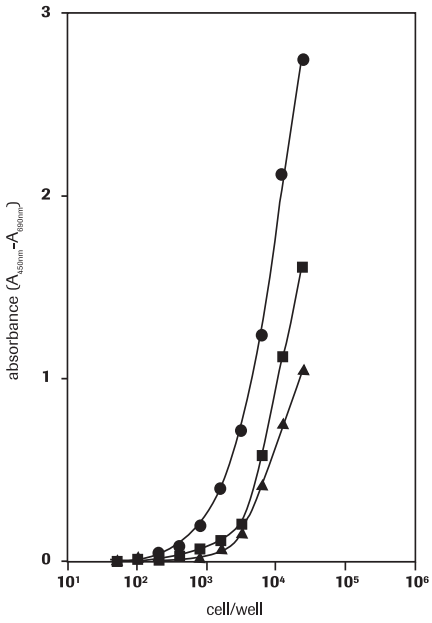

Fig. 5: Comparison of MTT (▲), XTT (■) and Cell Proliferation Reagent WST-1 (●). P815 cells at cell concentrations indicated in the figure were preincubated for 20 h before the addition of the various tetrazolium salts. After 4 h substrate reaction the absorbance was determined at the respective wavelength with an ELISA reader.

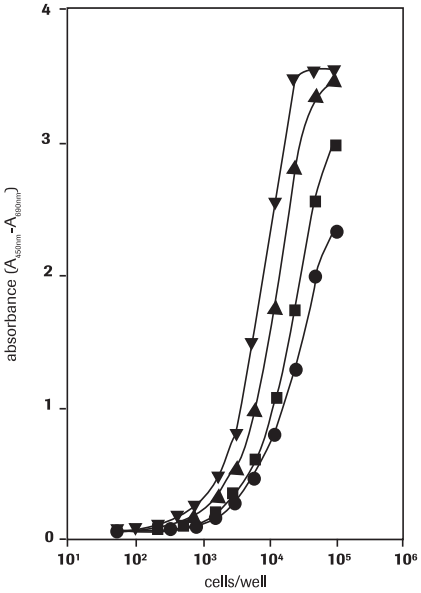

Fig. 6: Kinetics of the metabolism of the Cell Proliferation Reagent WST-1. A549 cells were cultured for 20 h at cell concentrations indicated in the figure, before the addition of Cell Proliferation Reagent WST-1. After 0.5 h (●, 1h (■), 2h (▲) and 4 h (▼) incubation periods the absorbance was determined by an ELISA reader.

References

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4. Supplementary Information

4.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this Instruction Manual:

Text Convention	Usage
Numbered stages labeled ①, ②, etc.	Stages in a process that usually occur in the order listed.
Numbered instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols

In this Instruction Manual, the following symbols are used to highlight important information:

Symbol	Description
🔍	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

4.2 Changes to Previous Version

Editorial changes

4.3 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, www.roche-applied-science.com and our Apoptosis and Cell Proliferation Special Interest Site:

http://www.roche-applied-science.com/apoptosis.

Product	Pack Size	Cat No.
BrdU labeling of proliferating cells *In situ* assay		
BrdU Labeling and Detection Kit I	1 kit (1000 test)	11 296 736 001
BrdU Labeling and Detection Kit II	1 kit (1000 test)	11 299 964 001
BrdU Labeling and Detection Kit III	1 kit (1000 test)	11 444 611 001
In Situ Cell Proliferation Kit, FLUOS	1 kit (1000 test)	11 810 740 001
Cell Proliferation ELISA, BrdU (colorimetric)	1 kit (1000 test)	11 647 229 001
Cell Proliferation ELISA, BrdU (chemiluminescent)	1 kit (1000 test)	11 669 915 001
Measurement of metabolic activity: Quantification in microplates		
Cell Proliferation Kit I (MTT)	1 kit (2500 test)	11 465 007 001
Cell Proliferation Kit II (XTT)	1 kit (2500 test)	11 465 015 001
Human TNF-α recombinant (*E.coli*)	10 µg (1 000 000 U)	11 371 843 001
Human TNF-α recombinant (yeast)	10 µg (1 000 000 U)	11 088 939 001
Interleukin-2, human (hIL-2) recombinant (*E.coli)*	10 000 U (5 µg; 50 ml)	10 799 068 001
	10 000 U (5 µg; 1 ml)	11 011 456 001
	50 000 U (25 µg; 5 ml)	11 147 528 001
Antibiotics for cell culture		
Penicillin/Streptomycin	for 20 ml (500x)	11 074 440 001
Single reagents for *in situ* assays and ELISA applications		
Anti-BrdU Formalin Grade	50 µg (500 µl)	11 170 376 001
Anti-BrdU-Fluorescein, Formalin Grade	50 µg (500 µl)	11 202 693 001
Anti-BrdU-Peroxidase, Fab fragments, Formalin Grade	15 U	11 585 860 001

Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site** at:

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country. Country-specific contact information will be displayed. Use the Product Search function to find Pack Inserts and Material Safety Data Sheets.

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