

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



Cell Proliferation Reagent WST-1

 **Version: 18**

Content Version: January 2021

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

Cat. No. 05 015 944 001	8 ml 800 tests
Cat. No. 11 644 807 001	25 ml 2,500 tests

Store the product at -15 to -25°C .

1.	General Information	3
1.1.	Contents	3
1.2.	Storage and Stability	3
	Storage Conditions (Product)	3
1.3.	Additional Equipment and Reagent required	4
1.4.	Application	4
2.	How to Use this Product	6
2.1.	Before you Begin	6
	Control Reactions	6
	Control (blank).....	6
	General Considerations.....	6
	Determination of optimal incubation periods	6
	Incubation requirements for high sensitivity	6
	Initial incubation.....	7
	Background absorbance.....	7
	Comparison of WST-1 to other cell proliferation agents.....	7
2.2.	Protocols	8
	Assay protocol	8
	Measurement of the proliferation of CTLL-2 cells after IL-2 stimulation	8
	Measurement of the cytotoxic effect of human tumor necrosis factor- α (hTNF- α) on WEHI-164 cells.....	9
2.3.	Parameters	10
	Working Concentration	10
3.	Additional Information on this Product	11
3.1.	Test Principle	11
	Overview of the basic steps.....	11
	Background information	11
4.	Supplementary Information	12
4.1.	Conventions.....	12
4.2.	Changes to previous version.....	12
4.3.	Ordering Information.....	12
4.4.	Trademarks.....	13
4.5.	License Disclaimer.....	13
4.6.	Regulatory Disclaimer.....	13
4.7.	Safety Data Sheet.....	13
4.8.	Contact and Support.....	13

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Catalog Number	Content
1	blue	Cell Proliferation Reagent WST-1	<ul style="list-style-type: none"> Read-to-use solution; clear and slightly red. Solution contains WST-1 and an electron coupling reagent diluted in phosphate-buffered saline (PBS). Filtered through 0.2 µm pore size membrane. 	11 644 807 001	1 bottle, 25 ml
				05 015 944 001	1 bottle, 8 ml

1.2. Storage and Stability

Storage Conditions (Product)

Shipped on dry ice.

When stored at –15 to –25°C, the product is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	blue	Cell Proliferation Reagent WST-1	<p>Store at –15 to –25°C.</p> <p>⚠ Keep protected from light.</p> <p>i <i>If precipitates or turbidity are observed upon thawing, warm up the solution to +37°C for 2 to 10 minutes and agitate to dissolve the precipitates. Do not centrifuge as the working concentration will decrease. The WST-1 reagent can be used without any limitations.</i></p> <p>Once thawed, store at +2 to +8°C for up to four weeks, protected from light. If the solution becomes viscous, warm up to +37°C for 2 to 10 minutes.</p> <p>For long-term storage, store in aliquots at –15 to –25°C.</p>

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- +37°C incubator
- Humidified chamber
- Centrifuge with rotor for microplates (for suspension cells only)
- ELISA reader for microplates. The wavelength to measure the absorbance of the formazan product is between 420 and 480 nm; the reference wavelength should be >600 nm.
- Multichannel pipettes (10 µl, 50 µl, and 100 µl)
- Sterile pipette tips
- Flat-bottomed 96-well microplates, tissue-culture grade

For the measurement of the proliferation of CTLL-2 cells after IL-2 stimulation

- Culture medium, such as RPMI containing 10% heat inactivated FCS (fetal calf serum), 2 mM L-glutamine, 1 mM Na-pyruvate, 1x non-essential amino acids, and 50 µM 2-mercaptoethanol
 - ⓘ *If an antibiotic will be used, supplement media with Penicillin-Streptomycin* or gentamicin.*
- Interleukin-2, human (hIL-2)* (10,000 U/ml, 5 µg/ml), sterile filtered

For the measurement of the cytotoxic effect of human tumor necrosis factor-α (hTNF-α) on WEHI-164 cells

- Culture medium, such as RPMI 1640 containing 10% heat inactivated FCS (fetal calf serum), 2 mM L-glutamine, and 1 µg/ml actinomycin C1 (actinomycin D)
 - ⓘ *If an antibiotic will be used, supplement media with Penicillin-Streptomycin* or gentamicin.*
- Tumor necrosis factor-α, human (hTNF-α)* (10 µg/ml), sterile filtered

1.4. Application

The Cell Proliferation Reagent WST-1 is designed for the nonradioactive, spectrophotometric quantification of cell proliferation, growth, viability, and chemosensitivity in cell populations using the 96-well microplate format. Applications include the:

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

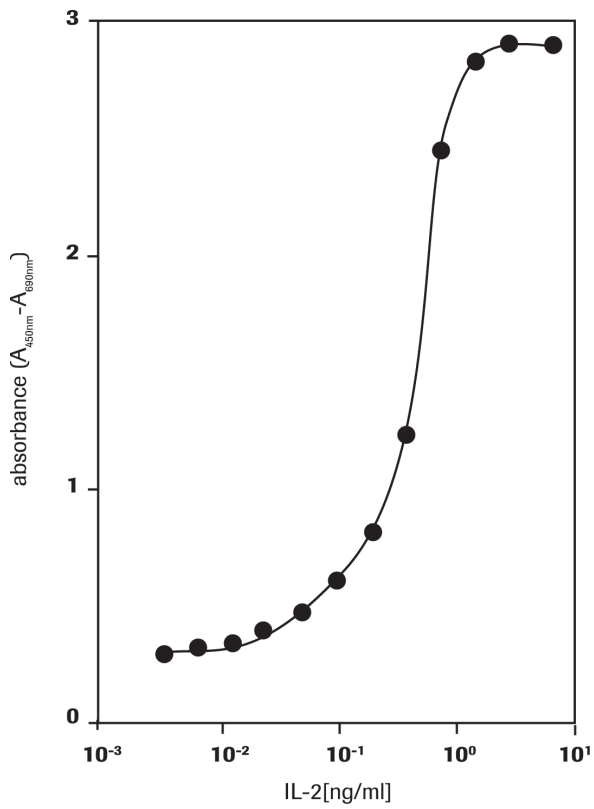


Fig. 1: Measurement of proliferation of CTLL-2 cells in response to human IL-2.

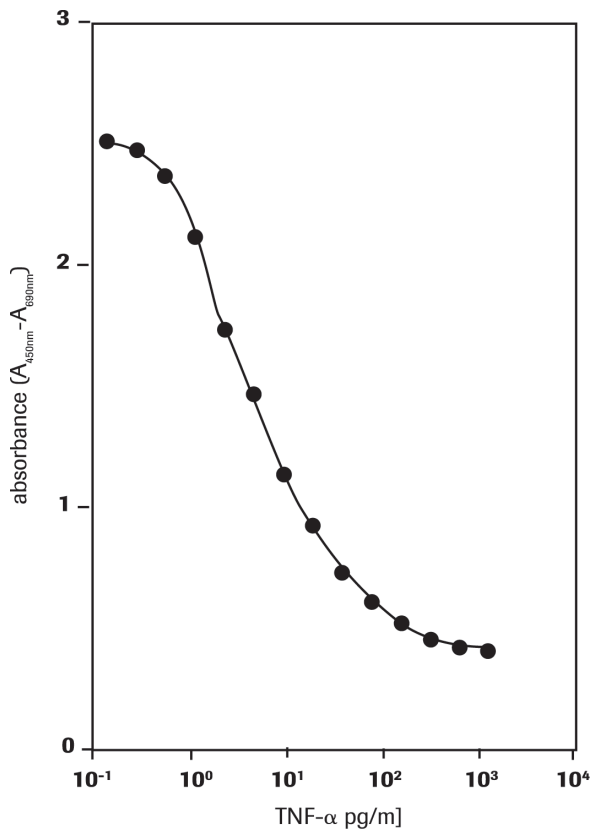


Fig. 2: Determination of the cytotoxic activity of human TNF- α on WEHI-164 cells.

2. How to Use this Product

2.1. Before you Begin

Control Reactions

Control (blank)

Add the same volume of culture medium and Cell Proliferation Reagent WST-1 as used in the experiment into one well, for example, 100 μ l culture medium plus 10 μ l 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.

General Considerations

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, for example, cell type and cell concentration used. Therefore, perform a preliminary experiment and measure the absorption repeatedly at different time points after the addition of the Cell Proliferation Reagent WST-1, for example, 0.5, 1, 2, and 4 hours. This allows you to determine the optimal incubation period for the particular experimental setup used (Fig. 3).

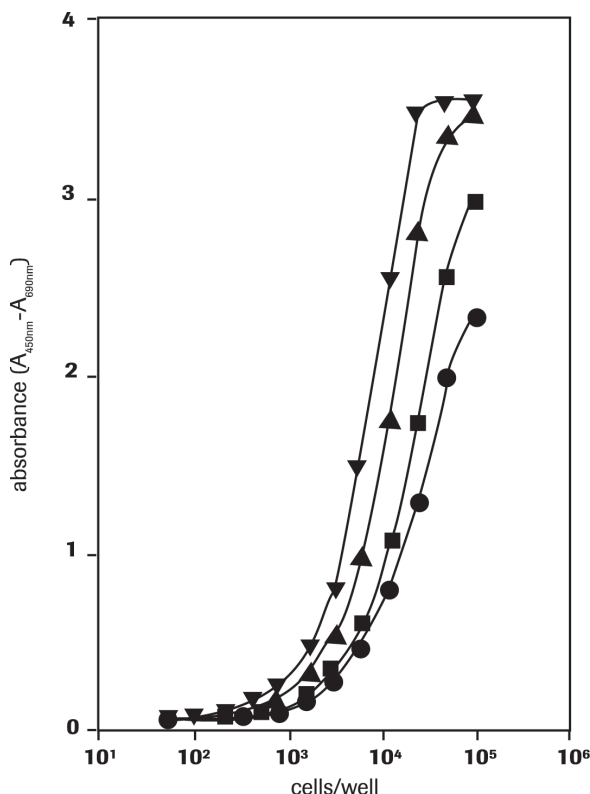


Fig. 3: Kinetics of the metabolism of the Cell Proliferation Reagent WST-1. A549 cells were cultured for 20 hours at cell concentrations indicated in the figure, before the addition of Cell Proliferation Reagent WST-1. After 0.5 hours (●), 1 hour (■), 2 hours (▲), and 4 hours (▼) incubation periods, the absorbance was determined by an ELISA reader.

Incubation requirements for high sensitivity

When high sensitivity is required, incubate the cells in the presence of Cell Proliferation Reagent WST-1 for longer periods of time (Fig. 3).

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, for example, 20 μl /well Cell Proliferation Reagent WST-1 cells are cultured in 200 μl /well culture medium.

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 hours is 0.1 to 0.2 absorbance units.

Comparison of WST-1 to other cell proliferation agents

The Cell Proliferation Reagent WST-1 (Figures 4 and 3) has several advantages compared to other cell proliferation reagents:

- In contrast to MTT, which is cleaved to a water-insoluble formazan crystal, and therefore has to be solubilized after cleavage, WST-1 yields water-soluble cleavage products similar to 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 (Figures 4 and 3).

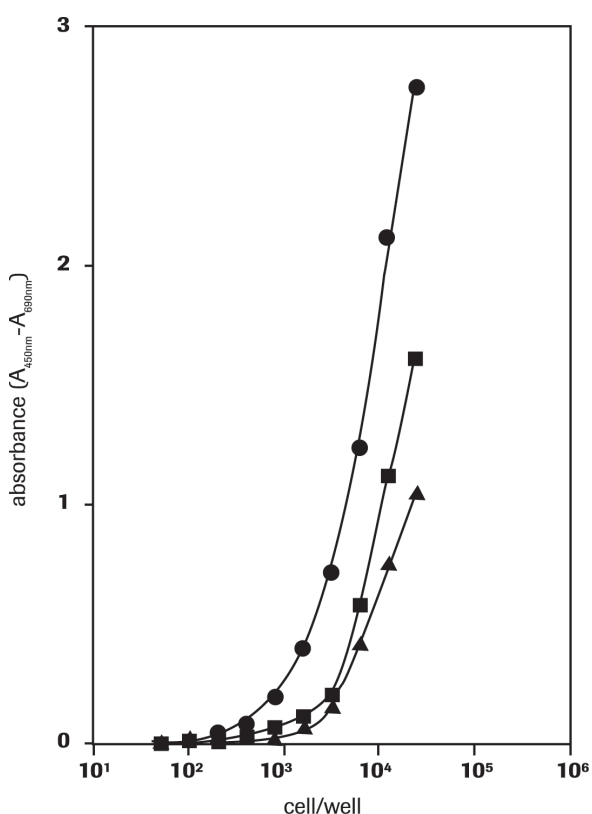


Fig. 4: Comparison of MTT (▲), XTT (■), and Cell Proliferation Reagent WST-1 (●). P815 cells at cell concentrations indicated in the figure were preincubated for 20 hours before the addition of the various tetrazolium salts. After 4 hours substrate reaction, the absorbance was determined at the respective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

2.2. Protocols

Assay protocol

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

i For most experimental setups, use a cell concentration of 0.1 to 5×10^4 /well and an incubation time of 24 to 96 hours.

The general assay protocol is shown below:

- 1 Grow cells in microplates (tissue-culture grade, 96 wells, flat bottom) in a final volume of 100 μ l/well culture medium in a humidified atmosphere, such as +37°C, 5 to 6.5% CO₂.

- 2 Add 10 μ l/well Cell Proliferation Reagent WST-1.

- 3 Incubate the cells for 0.5 to 4 hours in a humidified atmosphere, such as +37°C, 5 to 6.5% CO₂.

- 4 Shake thoroughly for 1 minute on a shaker.

- 5 Measure the absorbance of the samples against a background control as blank using a microplate (ELISA) reader at 420 to 480 nm. Use a reference wavelength >600 nm.

Measurement of the proliferation of CTLL-2 cells after IL-2 stimulation

For the determination of the activity of human Interleukin-2 (IL-2) on the mouse T-cell line CTLL-2, see Figure 1, section, **Application**.

- 1 Seed CTLL-2 cells at a concentration of 4×10^3 cells/well in 100 μ l culture medium containing various amounts of IL-2 (final concentration approximately 0.005 to 25 ng/ml) into microplates (tissue-culture grade, 96 wells, flat bottom).

- 2 Incubate cells for 48 hours at +37°C and 5 to 6.5% CO₂.

- 3 Add 10 μ l/well Cell Proliferation Reagent WST-1 and incubate for 4 hours at +37°C and 5 to 6.5% CO₂.

- 4 Shake thoroughly for 1 minute on a shaker.

- 5 Measure the absorbance of the samples against a background control as blank using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product is between 420 and 480 nm (maximum absorption at approximately 440 nm) according to the filters available for the ELISA reader used (Fig. 5). Use a reference wavelength >600 nm.

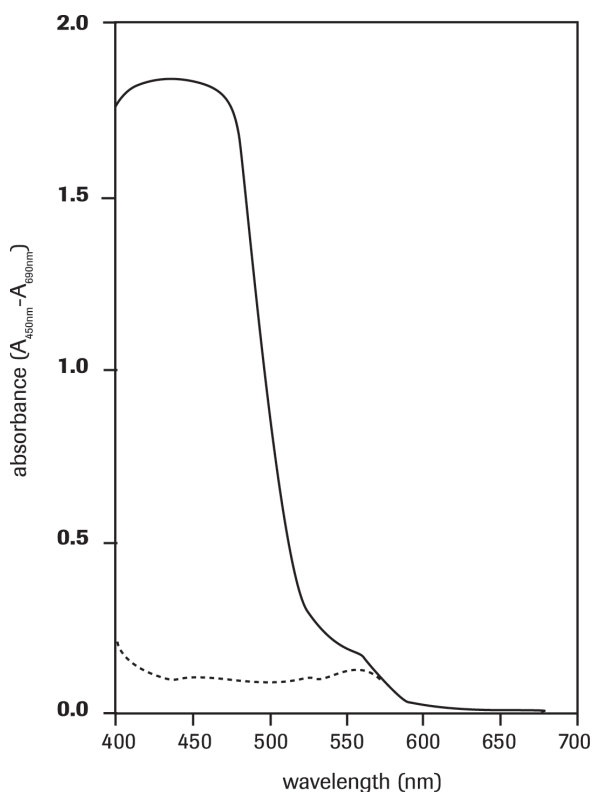


Fig. 5: Absorbance spectra of Cell Proliferation Reagent WST-1 (dotted line) and the reaction product formazan (solid line) after cleavage by mitochondrial dehydrogenase activity. The Cell Proliferation Reagent WST-1 was diluted 1:10 in a cell suspension in RPMI 1640 containing 10% FCS.

Measurement of the cytotoxic effect of human tumor necrosis factor- α (hTNF- α) on WEHI-164 cells

For the determination of the cytotoxic effect of hTNF- α on the mouse fibrosarcoma cell line WEHI-164, see Figure 2, section, **Application**.

- 1 Culture cells in microplates (tissue-culture grade, 96 wells, flat bottom) in a final volume of 100 μ l/well culture medium in a humidified atmosphere, such as +37°C and 5 to 6.5% CO₂.

- 2 Seed cells at a concentration of 5×10^4 cells/well in 100 μ l culture medium containing 1 μ g/ml actinomycin C1 and various amounts of hTNF- α (final concentration approximately 0.001 to 0.5 ng/ml) into microplates (tissue-culture grade, 96 wells, flat bottom).

- 3 Incubate cell cultures for 24 hours at +37°C and 5 to 6.5% CO₂.

- 4 After the incubation period, add 10 μ l of the Cell Proliferation Reagent WST-1 to each well.

- 5 Incubate the microplate for 4 hours in a humidified atmosphere, such as +37°C and 5 to 6.5% CO₂.

- 6 Shake thoroughly for 1 minute on a shaker.

- 7 Measure the absorbance of the samples against a background control as blank using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product is between 420 and 480 nm (maximum absorption at approximately 440 nm) according to the filters available for the ELISA reader used (Fig. 2, section, **Application**). Use a reference wavelength >600 nm.

2.3. Parameters

Working Concentration

Reagent	Working Concentration
Cell Proliferation Reagent WST-1	Add 10 µl/well to cells cultured in 100 µl/well (1:10 final dilution). <i>i</i> One vial will be sufficient to perform the 2,500 tests (25 microplates).
	Add 20 µl/well to cells cultured in 200 µl/well. <i>i</i> Use when for the initial incubation of cells, an increased volume of culture medium is required.

3. Additional Information on this Product

3.1. Test Principle

The assay is based on the cleavage of the slightly red tetrazolium salt WST-1 to form a dark red formazan dye by metabolically active cells (Fig. 6). Therefore, this conversion only occurs in viable cells. The formazan dye formed is soluble in aqueous solutions and is directly quantified using a scanning multiwell spectrophotometer (ELISA reader). This ensures a high degree of accuracy, enables online computer processing of the data (data collection, calculation, and report generation) and, thereby, enables the rapid and convenient handling of a high number of samples.

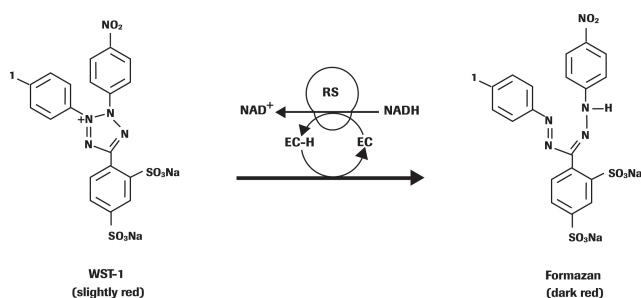


Fig. 6: Cleavage of the tetrazolium salt WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) to formazan.

EC = electron coupling reagent.

RS = mitochondrial succinate-tetrazolium-reductase system.

Overview of the basic steps

- 1 Cells grown in a 96-well tissue-culture plate are incubated with the slightly red WST-1 solution (1:10 final dilution) for 0.5 to 4 hours, see Figure 6.

- 2 After this incubation period, a dark red formazan solution is formed.

- 3 The solubilized formazan product is spectrophotometrically quantified using an ELISA reader.
 - An increase in the number of living cells results in an increase in the overall activity of mitochondrial dehydrogenases in the sample. This increase directly correlates to the amount of dark red formazan formed, as monitored by the absorbance (Fig. 4).

Background information

The determination of cellular proliferation, viability, and activation are key areas in a wide variety of cellular biological approaches. The need for sensitive, quantitative, reliable, and automated methods led to the development of standard assays. Such an example is based on the capability of the cells to incorporate a radioactively labeled substance, such as [3H] thymidine, or to release a radioisotope, such as [⁵¹Cr] after cell lysis. Alternatively, the incorporation of 5-bromo-2-deoxyuridine (BrdU)* in place of thymidine is monitored as a parameter for DNA synthesis and cellular proliferation in immunohistochemistry, in a cell ELISA, and FACS analysis. Cell proliferation and viability assays are of particular importance for routine applications. Tetrazolium salts, such as MTT, XTT, and WST-1 are especially useful for assaying the quantification of viable cells, because they are cleaved to form a formazan dye (Fig. 4; for UV absorbance spectrum, see Figure 5) only by metabolically active cells.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols

i *Information Note: Additional information about the current topic or procedure.*

⚠ Important Note: Information critical to the success of the current procedure or use of the product.

① ② ③ etc. Stages in a process that usually occur in the order listed.

① ② ③ etc. Steps in a procedure that must be performed in the order listed.

* (Asterisk) The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.

Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Tumor Necrosis Factor- α , human (hTNF- α)	1,000,000 U, 10 μ g, 1 ml	11 371 843 001
Interleukin-2, human (hIL-2)	10,000 U, 5 μ g, 50 ml	10 799 068 001
	10,000 U, 5 μ g, 1 ml	11 011 456 001
	50,000 U, 25 μ g, 5 ml	11 147 528 001
Penicillin-Streptomycin	for 20 ml, 500x	11 074 440 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:

List of biochemical reagent products.

4.6. Regulatory Disclaimer

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

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

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

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

