Immunservice

WST-1 Cell Proliferation Kit (ready-to-use)

USER MANUAL

1. Introduction

In life science research the measurement of cell proliferation and cell viability has become a key technology. Immunservice's WST-1 Cell Proliferation Kit (ready-to-use) provides an easy, fast and accurate method to determine cell proliferation, cell viability and cytotoxicity.

The assay is highly convenient and designed to enable a nonradioactive, spectrophotometric quantification in a single 96 well microtiter plate.

The intended applications for this product comprise:

- Measurement of proliferative response of various cell types to cytokines, growth factors, mitogens, nutrients, etc.
- Analysis of the impact of cytotoxic and cytostatic substances such as drugs or other pharmaceutical agents
- Screening of cell growth inhibitors, e.g. antibodies, physiological mediators, etc.

Assay Principle:

Quantification of cell proliferation is based on cleavage of the water-soluble tetrazolium salt WST-1 to the formazan dye by cellular mitochondrial dehydrogenase enzymes. These enzymes are active only in viable cells. An increase in the number of viable cells leads to an increase in the activity of mitochondrial dehydrogenases and accordingly to an increase formazan dye produced. The dark yellow colored formazan dye produced can be quantified by determining the absorbance at 420-480 nm with a microtiter plate reader. The absorbance strongly correlates with the number of metabolically active and viable cells in the sample.

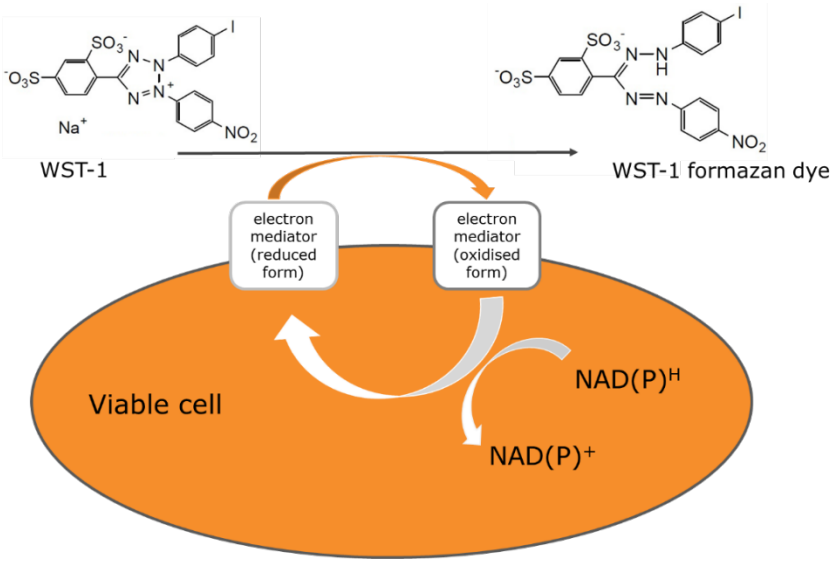

Figure 1: Cleavage of the tetrazolium salt WST-1 to WST-1 formazan dye by viable cells

2. Assay Overview

Quick protocol:

5 simple steps to the solution for your proliferation experiments:

1. Culture cells on microtiter plate.
2. Add WST-1 reagent from the ready-to-use Cell Proliferation Kit.
3. Incubate for 1 – 4 h.
4. Measure absorbance at 420 – 480 nm.
5. Analyze data.

Advantages at a glance:

Easy to use	○ Immunservice's WST-1 Kit is ready-to-use with easy to follow instructions. ○ A complete assay can be done on a single microtiter plate. ○ No need for washing, harvesting or solubilization of cells
Safe	○ Nonradioactive assay
Accurate & Sensitive	○ The absorbance strongly correlates with the number of viable cells.
Fast	○ Fast color development ○ Short incubation time ○ Processing of a large number of samples at the same time
Cost-effective	○ Our pricing is intended to provide the least expensive WST-1 Kit on the market. Immunservice's prices are lower than the competition's!
Stable	○ Immunservice's WST-1 Kit is stable for 6 months at -20°C without significant degradation.

3. Components and storage conditions

Cat. No	Components / Quantity	Storage
WST1.150813.1	WST-1 reagent and electron mediator in buffer solution / 10 ml (sufficient for 1000 assays)	-20°C
WST1.150813.2	WST-1 reagent and electron mediator in buffer solution / 25 ml (sufficient for 2500 assays)	-20°C
WST1.150813.3	WST-1 reagent and electron mediator in buffer solution / 4 x 25 ml (sufficient for 10000 assays)	-20°C

WST-1 Cell Proliferation Kit (ready-to-use) contains a clear, red solution. It should be stored at -20°C protected from light. To avoid repeated freezing and thawing, aliquot THE solution. Aliquots of 1 ml are sufficient for one 96-well microtiter plate. If stored properly, Immunservice WST-1 Cell Proliferation Kit (ready-to-use) is stable for at least 6 months.

Precipitates, turbidity or an increase in viscosity may occur, especially if stored at temperatures higher than -20°C (e.g., at +2°C to +8°C). If you observe precipitates, increased viscosity or turbidity, heat the solution to 37°C for several minutes and gently mix to dissolve. Do not centrifuge the solution. This would lead to a loss of the proper working concentration.

Additional equipment required:

- Standard laboratory equipment (incubator, centrifuge, pipettors, pipette tips, etc.)
- 96-well microtiter spectrophotometer (ELISA plate reader) with filter for wavelengths between 420 – 480 nm. For subtracting a reference wavelength, a filter above 600 nm is recommended.
- 96-well microtiter plate

4. Assay Protocol

Tips before starting:

Working concentration of WST-1 Cell Proliferation Kit (ready-to-use)

Add 10 µl/well WST-1 reagent from WST-1 Cell Proliferation Kit (ready-to-use) to cells cultured in 100 µl/well culture medium. If cells are cultured in other amountS of medium, adjust the WST-1 solution accordingly. For example, use 20 µl/WST-1 when culturing in 200 µl/well medium.

Incubation time

The appropriate incubation time depends on the individual experimental setup used. We recommend determining the optimal incubation time for the specific experimental setup in a preliminary experiment. For this purpose plates can be read by the microtiter plate reader and returned to the incubator for further color development several times at different time points (e.g. after 1, 2, 3 and 4 h).
If high sensitivity is required, incubation for longer periods of time may be necessary.

Controls

Background control (blank control):
It is recommended to use a background control (blank control). To do so, add the same volume of culture medium and WST-1 Cell Proliferation ready-to-use solution as used in the experiment to one well (e.g. 100 µl culture medium + 10 µl WST-1). Do not add cells to the blank control well. Set this background control well as a blank position for the microtiter plate reader.
Note:
The background control absorbance depends on the culture medium, the incubation time and exposure to light. Typical background absorbance after 2 hours is between 0.1 - 0.2 absorbance units.

Negative/Positive Control:
If possible, depending on your specific experiment, set up appropriate positive and negative control wells.

Cell Proliferation Assay Protocol:

1. Culture cells (0.1-5 x 10^4/well) on a 96-well microtiter plate (tissue culture grade, flat bottom) in a final volume of 100 µl/well culture medium in an incubator (37°C, 5% CO_2, humidified atmosphere).

Note:

For most experiments, a cell concentration between 0.1-5 x 10^4 cells/well is appropriate. For toxicity assays, the use of more cells may be advantageous (e.g. 5 x 10^4 – 5 x 10^5 cells/well).

2. Incubate cells for 24-96 hours.

Note:

Determine the optimal incubation time for the particular experimental setup used.

3. Add 10 µl of WST-1 reagent from the Kit to each well.

Note:

Adjust the WST-1 volume accordingly if the cells are cultured in different volumes of culture medium (e.g. 20µl WST-1 to 200 µl culture medium).

4. Incubate cells for 1-4 hours under standard culture conditions (37°C, 5% CO_2, humidified atmosphere).

Note:

Determine the optimal incubation time for the particular experimental setup used.

5. Shake the microtiter plate thoroughly for 1 minute on a shaker.

6. Measure the absorbance of the plate against a blank control using a microtiter plate reader. A wavelength between 420-480 nm (optimal absorbance at 440 nm) is appropriate to measure the formazan dye produced. The reference wavelength should be > 600 nm.

Assay notes:

1. Measure samples in duplicate or triplicate to ensure the accuracy of results.
2. The assay can be stopped by adding 10 µl of 1% sodium dodecyl sulfate (SDS) to each well.
3. Phenol Red in the culture medium does not significantly interfere with the reading.

5. Ordering information

Please order online via our homepage, by telephone, fax or email.

Homepage: http://www.immunservice.com
Telephone: +49 (0)40 / 611 35 184
Fax: +49 (0)40 / 380 178 572 79
Email: order@immunservice.com

Please do not hesitate to contact us to ask questions, suggest improvements or report new applications.

6. Related Products

1.	WST-1 CTLL-2 Cell Proliferation Kit	Cat.No. WST1.CTLL150813.1 Cat.No. WST1.CTLL150813.2 Cat.No. WST1.CTLL150813.3
2.	i2cult - Complete Medium for IL-2-dependend cells	Cat.No. IL2.130411.4
3.	i2cult - CTLL-2 Complete Medium	Cat.No. IL2.130411.5
4.	Recombinant Human Interleukin-2 (rhIL-2)	Cat.No. IL2.130411.1 Cat.No. IL2.130411.2 Cat.No. IL2.130411.3

For a complete overview of related products and user manuals, please visit and bookmark our homepage http://www.immunservice.com.

7. References

1. Berridge, M. V., Herst, P. M. & Tan, A. S. Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. Biotechnol. Annu. Rev. 11, 127–152 (2005)

2. Cook, J. A. & Mitchell, J. B. Viability measurements in mammalian cell systems. Anal. Biochem. 179, 1–7 (1989)

3. Heiden, M. G. Vander et al. The Metabolic Requirements of Cell Proliferation. 324, 1029–1034 (2009).

4. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63 (1983).

For additional references, please contact Research@immunservice.com.

www.immunservice.com

For technical questions, please do not hesitate to contact us by email: support@immunservice.com

Immunservice GmbH
Christoph-Probst-Weg 4
20251 Hamburg, Germany
Tel.: +49 (0)40 / 611 35 184
Fax: +49 (0)40 / 380 178 572 79