Immunservice

WST-1 CTLL-2 cell proliferation Kit (ready-to-use)

Optimized for applications with CTLL-2 cells

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 CTLL-2 cell proliferation Kit provides an easy, fast and accurate method to determine cell proliferation, cell viability and cytotoxicity.

The Kit was specifically optimized for applications with CTLL-2 cells. The cytotoxic murine T-cell line CTLL-2 is well-suited and frequently used to assess the biological activity of the cytokines Interleukin 2 (IL-2), as well as IL-4, IL-12 and IL-18, via the extent of cell proliferation.

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

The intended applications for this product comprise:

- Measurement of CTLL-2 cell proliferation.
- Determination of the biological activity of human and murine IL-2 and IL-4, as well as murine IL-12 and IL-18 via measurement of CTLL-2 cell proliferation.
- Impact of cytotoxic and cytostatic substances (drugs, other pharmaceutical agents).
- Immune checkpoint inhibitory effects
- Screening and specific detection of cell growth inhibitors, e.g. antibodies, physiological mediators, virus- and tumor inducing immune inhibitors.

Assay Principle:

The quantification of cell proliferation is based on the cleavage of the water soluble tetrazolium salt WST-1 to formazan dye by cellular mitochondrial dehydrogenase enzymes. These enzymes are active only in viable cells. Expansion in the number of viable cells leads to an increase in the activity of mitochondrial dehydrogenases and by association to an increase of produced formazan dye. The produced dark yellow colored formazan dye 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.

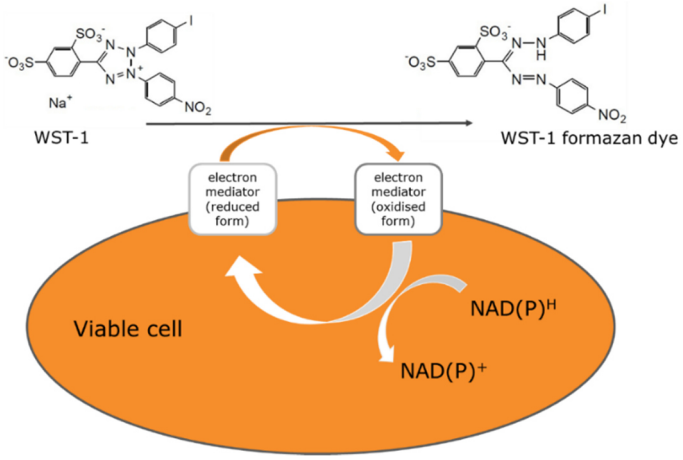

Figure 1: Cleavage of the tetrazolium salt WST-1 to WST-1 formazan dye by viable cells

2. Assay Overview

Quick protocol:

5 simple steps to the solution of your proliferation experiments:

1. Culture CTLL-2 cells in microtiter plate
2. Add WST-1 reagent from CTLL-2 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 CTLL-2 cell proliferation Kit is ready-to-use with easy to follow instructions ○ Complete assay can be done in a single microtiter plate ○ No need for washing, harvesting or solubilization of cells
Safe	○ Non-radioactive assay
Accurate & Sensitive	○ 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 cheapest WST-1 Kit on the market. Immunservice's prices beat the competition!
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 and Quantity	Storage
WST1.CTLL150813.1	WST-1 reagent and electron mediator in buffer solution / 10 ml (sufficient for 1000 assays)	-20°C
WST1.CTLL150813.2	WST-1 reagent and electron mediator in buffer solution / 25 ml (sufficient for 2500 assays)	-20°C
WST1.CTLL150813.3	WST-1 reagent and electron mediator in buffer solution / 4 x 25 ml (sufficient for 10000 assays)	-20°C

The WST-1 CTLL-2 cell proliferation kit (ready-to-use) contains a clear, red solution. It should be stored at -20°C, protected from light. To avoid repeated freeze-thaw cycles, aliquot solution. Aliquots of 1 ml are sufficient for one 96-well microtiter plate. If stored accordingly the Immunservice WST-1 CTLL-2 cell proliferation Kit is stable for at least 6 months.

The formation of precipitates, turbidity or viscosity is possible, especially if stored at temperatures higher than -20°C (f.e. at +2°C-+8°C). If you observe precipitates, viscosity or turbidity, warm up solution to 37°C for several minutes and gently mix to dissolve.

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 ready-to-use cell proliferation Kit

Add 10 µl/well of WST-1 reagent from WST-1 CTLL-2 cell proliferation solution (ready-to-use) to CTLL-2 cells cultured in 100 µl/well culture medium. If cells are cultured in other amount of medium, use 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 to determine 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, the incubation for longer periods of time may be feasible.

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 of WST-1 CTLL-2 cell proliferation Kit as used in the experiment into one well (e.g. 100 µl culture medium + 10 µl WST-1). Do not add cells into 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.

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

Cell Proliferation Assay Protocol:

1. Culture 4 x 10^3 CTLL-2 cells/well in 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 of 4 x 10^3 cells/well is appropriate. For toxicity assays, the use of more cells can 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 WST-1 Kit to each well.

Note:

Adjust WST-1 volume accordingly if the CTLL-2 cells are cultured in different volumes of culture medium (e.g. 20 µl 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 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 duplicates or triplicates to ensure accuracy of results.
2. The assay can be stopped by adding 10 µl of 1% Sodium dodecyl sulfate (SDS) into each well.
3. Phenol Red in 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
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Email: order@immunservice.com

Please don’t hesitate to contact us to ask questions, suggest enhancements or report new applications.

6. Related Products

1.	WST-1 ready to use cell proliferation Kit	Cat.No. WST1.150813.1 Cat.No. WST1.150813.2 Cat.No. WST1.150813.3
2.	i2cult - CTLL-2 Complete Medium	Cat.No. IL2.130411.5
3.	Recombinant Human Interleukin-2 (rhIL-2)	Cat.No. IL2.130411.1 Cat.No. IL2.130411.2 Cat.No. IL2.130411.3
4.	i2cult - Complete Medium for the cultivation of IL-2-dependend cells	Cat.No. IL2.130411.4

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

7. References

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

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

5. Khatri A1, Husaini Y, R. P. Murine CTLL-2 cells respond to mIL12: prospects for developing an alternative bioassay for measurement of murine cytokines IL12 and IL18. J. Immunol. Methods 326, 41–53 (2007)

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