User Manual

Premixed WST-1 Cell Proliferation Reagent

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I. Introduction

Cell proliferation and viability measurements are important tools for research in the life sciences. Many standard cell proliferation assays are based on measurements of radioactive nucleoside incorporation into DNA, since DNA synthesis accompanies cell proliferation. Nonradioactive cell proliferation assays have also been developed, based on the spectrophotometric detection of tetrazolium salts that are cleaved to colored formazan dyes by cellular enzymes.

Several tetrazolium salts, including MTT (Mossmann, 1983; Carmichael *et al.*, 1987; Vistica *et al.*, 1991), XTT (Scudiero, *et al.*, 1988; Weislow *et al.*, 1989; Roehm *et al.*, 1991) and MTS (Cory, *et al.*, 1991) have been used to assay cell proliferation and viability. These assays are based on tetrazolium salt cleavage to formazan-class dyes by mitochondrial succinate-tetrazolium reductase (Slater, *et al.*, 1963) which is present in viable cells. The total activity of this mitochondrial dehydrogenase increases proportionally to the number of viable cells, leading to an increase in tetrazolium salt conversion to formazan dye, which is in turn quantified by absorbance. However, the formazan dyes derived from most tetrazolium salts must be solubilized with organic solvents or surfactants before they can be quantified. In contrast, the formazan dye derived from the tetrazolium salt WST-1 is water soluble, simplifying the assay procedure and preserving your live cells.

The **Premixed WST-1 Cell Proliferation Reagent** provides a method to measure cell proliferation based on the enzymatic cleavage of the tetrazolium salt WST-1 to a water-soluble formazan dye (Figure 1) which can be detected by absorbance at 420-480 nm. The simple, one-solution assay saves time and is easy to use in a 96-well plate format. It simplifies time course experiments, since the results can be read repeatedly after the Premixed WST-1 Cell Proliferation Reagent is added to the samples. Furthermore, it improves safety by avoiding radioactive isotopes and organic solvents.

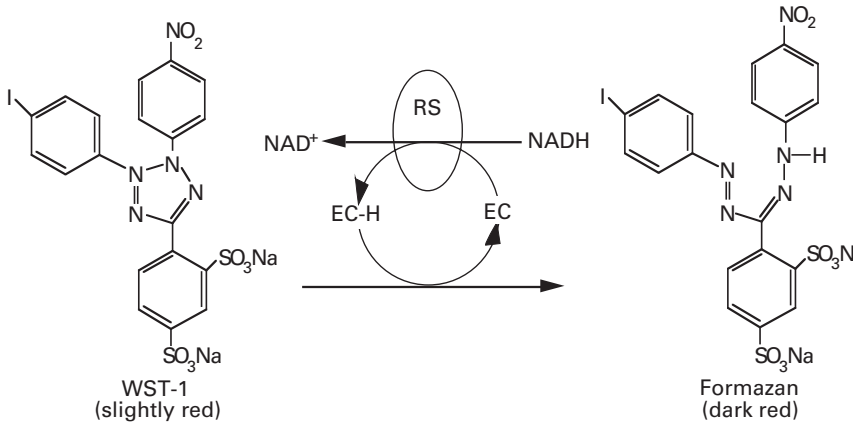

Figure 1. Cleavage of the tetrazolium salt WST-1 to Formazan. (EC = electron coupling reagent, RS = mitochondrial succinate-tetrazolium reductase system)

Premixed WST-1 Cell Proliferation Assay uses include:

- Measuring cell proliferation in response to growth factors, cytokines, mitogens, or nutrients.
- Analyzing cytotoxic and cytostatic compounds such as anticancer drugs and other pharmaceuticals.
- Evaluating physiological mediators and antibodies which inhibit cell growth.

The Premixed WST-1 Cell Proliferation Reagent provides several advantages over other cell proliferation assay reagents:

- **Safety:** No radioactive isotopes are required. Furthermore, the Premixed WST-1 Cell Proliferation Reagent is water soluble and does not require volatile organic solvents for solubilization.
- **Accuracy:** Absorbance strongly correlates to the number of viable cells.
- **Ease of Use:** The Premixed WST-1 Cell Proliferation Reagent is supplied as a ready-to-use, sterile solution. The entire assay can be performed in one 96-well plate, without washing, harvesting, or solubilization steps.
- **Flexibility:** Assay plates can be read and returned to the incubator several times.

II. List of Components

Store the unopened Premixed WST-1 Cell Proliferation Reagent at –20°C, protected from light.

Premixed WST-1 Cell Proliferation Reagent is a clear, slightly red, ready-to-use solution containing WST-1 and an electron coupling reagent, diluted in sterile phosphate buffered saline. If precipitates or turbidity are observed upon thawing, warm the solution to 37°C for 2–10 minutes and agitate to dissolve the precipitates. Centrifugation decreases the working concentration, and is not recommended. Once the Premixed WST-1 Cell Proliferation Reagent has been thawed, store at 4°C, protected from light, for up to several weeks. Please note that the solution may become viscous. For longer periods, store in aliquots at –20°C, protected from light. Do not freeze-thaw repeatedly.

The Premixed WST-1 Cell Proliferation Reagent contains 25 ml of reagent, enough for 2,500 reactions at 10 µl/reaction.

Premixed WST-1 Cell Proliferation Reagent (Cat. No. 630118)

- 25 ml Premixed WST-1 Cell Proliferation Reagent

Supporting Documents

- Premixed WST-1 Cell Proliferation Reagent User Manual (PT 3946-1)

Visit our web site at **www.clontech.com** for a current list of Cell Signaling products.

III. Additional Materials Required

The following materials are required but not supplied:

- Incubator (37°C)
- Shaker
- Multiwell plate reader with a filter for a wavelength between 420–480 nm. If a reference wavelength is to be subtracted, an additional filter above 600 nm is recommended.
- Multichannel pipettors (10, 50, 100 µl)
- Sterile pipette tips
- Flat bottomed 96-well tissue culture plates
- Microscope
- Hemacytometer

IV. Premixed WST-1 Cell Proliferation Assay Protocol

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING.

Protocol
1–4.5 hr.

A. Protocol: Measuring Cell Proliferation

1. Culture cells in flat bottomed 96-well plates in a final volume of 100 µl/well culture medium in a humidified atmosphere (e.g. 37˚C, 5% CO_2). Reserve one well as a background control (culture medium without cells).

   Note: The incubation period and cell density of the culture depend on the particular experimental conditions and on the cell line used. For most experimental setups, a cell density between 10^3 and 5 x 10^4 cells/well and an incubation time of 24 to 96 hr is appropriate.

2. Add 10 µl of Premixed WST-1 Cell Proliferation Reagent to each well (1:10 final dilution).

   Note: Premixed WST-1 Cell Proliferation Reagent should be used at a final dilution of 1:10. If cells are cultured in 200 µl culture medium, add 20 µl Premixed WST-1 Cell Proliferation Reagent per well.

3. Incubate the plate for 0.5 to 4 hr at 37˚C in a humidified atmosphere maintained at 5% CO_2.

4. Shake thoroughly for 1 minute on a shaker.

5. Measure the absorbance at 420–480 nm (maximum absorption is at ~440 nm), using a multiwell plate reader. The reference wavelength should be greater than 600 nm.

   Note: The absorbance level of the background control well (containing culture medium plus Premixed WST-1 Cell Proliferation Reagent, without cells) will depend on the culture medium, incubation time, and exposure to light. Typical background absorbance after 2 hr is between 0.1–0.2 absorbance units.

Protocol
1–4.5 hr.

B. Protocol: Optimizing Incubation Period

The appropriate incubation time after the addition of Premixed WST-1 Cell Proliferation Reagent depends on the individual experimental setup (e.g. cell type and cell density used). Therefore, we recommend that you perform a preliminary experiment where the absorbance is measured repeatedly at several time points (i.e., 0.5, 1, 2 and 4 hr) after the addition of the Premixed WST-1 Cell Proliferation Reagent. If higher sensitivity is required, incubate cells in Premixed WST-1 Cell Proliferation Reagent for longer periods of time.

V. References

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Appendix A: Example Applications

A. Cell Proliferation Assay

One application of the Premixed WST-1 Cell Proliferation Assay is to measure cell proliferation in response to growth factors, cytokines, mitogens or nutrients. In this example, the assay is used to measure the effect of human interleukin-2 (IL-2) on the mouse T cell line CTLL-2 (Figure 2).

Additional Reagents:

- RPMI 1640 culture medium, 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
- Mouse T cell line CTLL-2

Protocol Application:

1. Seed CTLL-2 cells at a density of 4 x 10^3 cells/well in 100 µl culture medium containing various amounts of IL-2 (final concentrations of 0.005–25 ng/ml) in flat bottom 96-well tissue culture plates. Reserve one well without cells for a background control (culture medium only).
2. Culture cells for 48 hr at 37˚C in a humidified atmosphere maintained at 5% CO_2.
3. Add 10 µl of Premixed WST-1 Cell Proliferation Reagent to each well.
4. Incubate for 4 hr at 37˚C and 5% CO_2.
5. Shake thoroughly for 1 minute on a shaker.
6. Measure the absorbance of the samples at 450 nm (reference wavelength 690 nm) against the background control, using a multiwell plate reader.

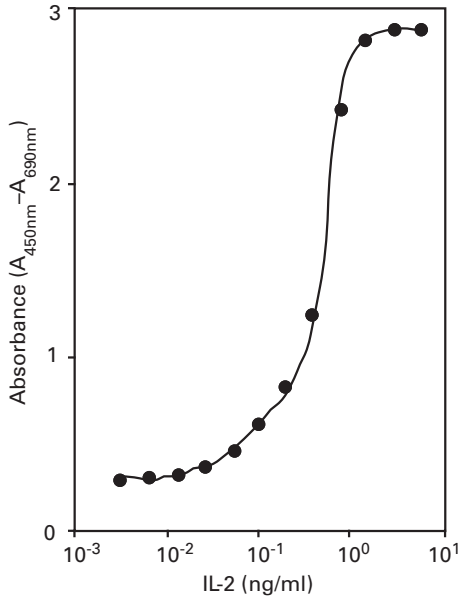

Figure 2. Cell proliferation measurements for CTLL-2 cells in response to human IL-2.

Appendix A: Example Applications continued

B. Cytotoxicity Assay

Another application of the Premixed WST-1 Cell Proliferation Assay is to assess viability in response to cytotoxic and cytostatic compounds. In this example, the assay is used to measure the cytotoxic effect of human tumor necrosis factor (TNF-α) on the mouse fibrosarcoma cell line WEHI-164 (Figure 3).

Additional Reagents:

- RPMI 1640 culture medium, 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 mg/ml), sterile
- Mouse fibrosarcoma cell line WEHI-164

Protocol Application:

1. Preincubate WEHI-164 cells at a density of 1 x 10^6 cells/ml in culture medium with actinomycin C1 (1 µg/ml) for 3 hr at 37°C and 5% CO_2.
2. Seed WEHI-164 cells at a density of 5 x 10^4 cells/well in 100 µl culture medium containing actinomycin C1 (1 µg/ml) and various amounts of TNF-α (final concentrations of 0.001–0.5 ng/ml) in flat bottom 96-well tissue culture plates. Reserve one well without cells for a background control (culture medium only).
3. Incubate the plate for 24 hr at 37°C in a humidified atmosphere maintained at 5% CO_2.
4. Add 10 µl of Premixed WST-1 Cell Proliferation Reagent to each well.
5. Incubate for 4 hr at 37°C and 5% CO_2.
6. Shake thoroughly for 1 minute on a shaker.
7. Measure the absorbance of the samples at 450 nm (reference wavelength 690 nm) against the background control, using a multiwell plate reader.

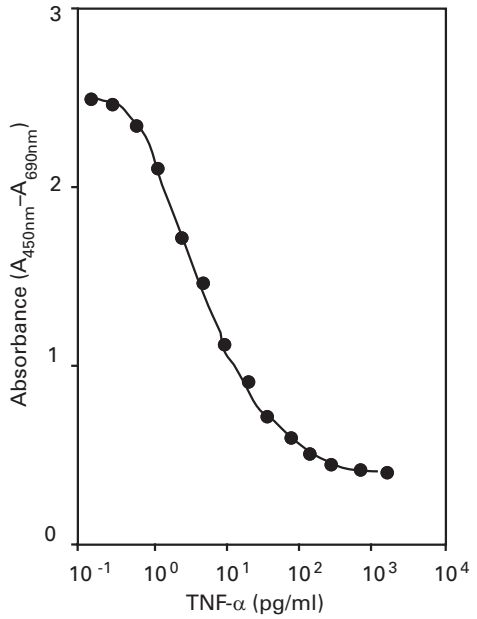

Figure 3. Measurement of the cytotoxic effect of human TNF-α on WEHI-164 cells.