Cat. # MK400

For Research Use

TaKaRa

Premix WST-1
Cell Proliferation Assay System

Product Manual

v201904Da

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I. Description

In life science reserch, it is often important to measure cell proliferation or cell viability. Some standard methods were developed to provide a sensitive, accurate, fast, and simple assay. In these methods, DNA synthesis is detected by the measuring uptake of RI-labeled nucleoside, as DNA synthesis can be used as an indirect measure of cell proliferation or cell viability.
Recently a new measuring method has emerged for the detection of cell proliferation or cell viability. In this new method, the number of viable cells are measured by the detection of cleaved tetrazolium salts that added into the medium. Washing and collecting cells is not required, and all procedures, from the culture in small scale to data analysis with a microplate reader, can be carried out in the same microtiter plate.
The Premix WST-1 enables cell proliferation and cell viability to be measured with a colorimetric assay, based on cleavage of tetrazolium salts by mitochondrial dehydrogenase in viable cells. This product takes the place of RI-labeled nucleoside, and provides a non-RI method for the analysis of cell proliferation or cell viability.

II. Principle

Recently, various kinds of tetrazolium salts (e.g. MTT[2 - 4], XTT[5 - 7], MTS[8], etc.) are available to measure cell proliferation or cell viability. These teterazolium salts are cleaved to formazan dye by succinate-tetrazolium reductase (EC 1.3.99.1)[9], which exists in the mitochondrial respiratory chain and is active only in viable cells. Total activity of this mitochondrial dehydrogenase in a sample rises with increased viable cells. As the increase of enzymes' activity leads an increase of the production of formazan dye, the quantity of formazan dye is related directly with the number of metabolically active cells in the medium. The formazan dye formed by metabolically active cell can be quantitated by measuring its absorbance by an ELISA reader, allowing measurement of cell proliferation activity and viability. The absorbance of formazan dye is in direct proportion to the number of viable cells. (Figure 1)
This product is also designed for non-radioactive quantification of cell growth and viability in proliferation and chemosensitivity assay.
It can be used for

- The measurement of cell proliferation, which is sensitive to growth factors, cytokines, mitogens, and nutrients.
- The analysis of cytotoxic and cytostatic compounds such as anticancer drugs, etc.
- The evaluation of physiological mediators and antibodies that inhibit cell growth.

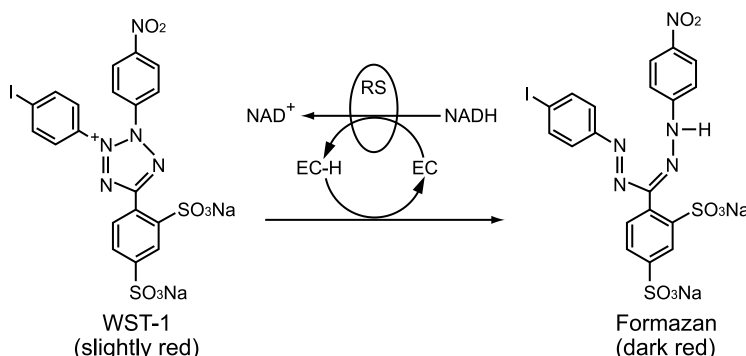

Figure1. Cleavage of the tetrazolium salt (WST-1) to fromazan. (EC=electron coupling reagent, RS=mitochondrial succinate-tetrazolium-reductase system)

III. Features

1. *Safety* : No radioactive isotopes are required
   No volatile organic solvent is required for solubilization.
2. *Accuracy* : Absorbance strongly correlates with the number of viable cells.
3. *Sensitivity* : More sensitive than using MTT.
   As sensitive as XTT.
4. *Ease of use* :
   - Supplied as a ready-to-use, sterile solution.
   - Short reaction time.
   - The entire assay is performed in one microtiter plate.
   - No need for washing, harvesting or solubilization
     Processing a large number of samples is possible by using a multiwell-ELISA reader.
     No isotope disposal and radiation safety paperwork.
5. *Flexibility* : Plates can be read and returned several times to the incubator for further color development.

IV. Components (For 2,500 tests)

Premix WST-1 25 ml

Note : The Premix WST-1 is a ready-to-use solution containing WST-1 and an electron coupling reagent, diluted in sterile phosphate buffered saline.
It is recommended that 10 μl/well Premix WST-1 is added to cells cultured in 100 μl/well (1 : 10 final dilution). If cells are cultured in 200 μl/well, add 20 μl/well Premix WST-1. Using 100 μl/well cell culture volume, one bottle will contain sufficient volume to perform 2,500 tests (25 microtiter plates).

V. Storage

・Stored at -20℃ (protected from light)

Note : If a precipitate is observed when dissolving this reagent, redissolve any precipitate by warming at 37℃ for several minutes with gentle shaking.
Once thawed, it can be stored at 4℃, protected from light, for several weeks.
For longer storage, it is recommended to store in aliquots at -20℃.
Do not repeatedly freeze and thaw.

VI. Protocol

1. Materials required by not provided

- Incubator (37℃)
- Centrifuge
- Microtiter plate (ELISA) reader with a filter for the use at a wavelength between 420 - 480 nm (if a reference wavelength is to be substracted, a filter for above 600 nm is recommended.)
- Microscope
- Hemacytometer
- Multichannel pipettor (10, 50, and 100 μl)
- Sterile pipette tips
- 96-well microtiter plates (cell culture grade, flat bottom)

2. Protocol

1. Culture cells in microtiter plates (tissue culture grade, 96 wells, flat bottom) in a final volume of 100 μl/well culture medium in a humidified atmosphere (e.g. 37℃ 5% CO_2).
   * 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 concentration between 0.1 and 5 x 10^4 cells/well and an incubation time of 24 to 96 hrs is appropriate.
2. After the incubation period, add 10 μl/well Premix WST-1.
3. Incubate the cells for 0.5 to 4 hrs in a humidified atmosphere (e.g. 37℃ 5% CO_2).
4. Measure the absorbance of the samples against a background control (see VI-3) as a blank using a microtiter plate (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. The reference wavelength should be more than 600 nm.

   Note 1: The appropriate incubation time after the addition of Premix 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 Premix WST-1 (e.g. 0.5, 1, 2, and 4 hrs) in a preliminary experiment.

   Note 2: If high sensitivity is required, incubate the cells in the presence of Premix WST-1 for longer periods of time.

3. Background control (blank)

Add the same volume of culture medium and Premix WST-1 as used in the experiment into one well (e.g. 100 μl culture medium plus 10 μl Premix WST-1). Use this background control (absorbance of culture medium plus Premix WST-1 in the absence of cells) as a blank position for the ELISA reader.
Slight spontaneous absorbance occurs if Premix 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 hrs is between 0.1 - 0.2 absorbance units.

VII. Application

1. Cell proliferation assay (IL-2)

Determination of the effect of human interleukin-2 (IL-2) on the mouse T cell line CTLL-2.

[Reagents]

- Culture medium, e.g.
  RPMI 1640 Medium
  10% heat-inactivated fetal calf serum (FCS)
  2 mM L-glutamine
  1 mM Na-pyruvate
  1X non-essential amino acids
  50 μM 2-mercaptoethanol
  * Penicillin/streptomycin or gentamicin (optional)
- Human IL-2 (10,000 U/ml; 5 μg/ml), sterile
- Premix WST-1

[Procedure]

1. Seed CTLL-2 cells at a concentration of 4 x 10^3 cells/well in 100 μl culture medium containing various amounts of IL-2 (final concentration 0.005 - 25 ng/ml) in microtiter plates (tissue culture grade, 96 wells, flat bottom).
2. Incubate cells for 48 hrs at 37℃ and 5% CO_2.
3. Add 10 μl/well Premix WST-1 and incubate for 4 hrs at 37℃ and 5% CO_2.
4. Measure the absorbance as described in the protocol (VI - 2).

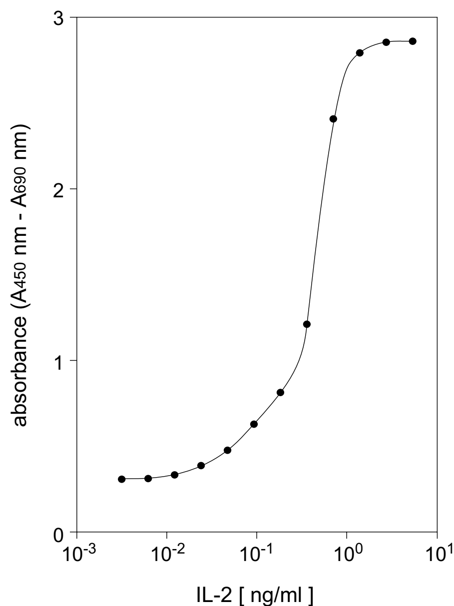

Figure 2. Meaurement of cell proliferation of CTLL-2 cell in the presence of human IL-2

2. Cytotoxicity assay (TNF-α)

Determination of the cytotoxic effect of human tumor necrosis factor-α (TNF-α) on the mouse fibrosarcoma cell line WEHI-164.

[Reagents]

- Culture medium, e.g.
  RPMI 1640 Medium
  10% heat-inactivated FCS
  2 mM L-glutamine
  1 μg/ml actinomycin C1 (actinomycin D)
  * Penicillin/streptomycin or gentamicin (optional)
- human TNF-α (10 μg/ml), sterile
- Premix WST-1

[Procedure]

1. Preincubate WEHI-164 cells at a concentration of 1 x 10^6 cells/ml in culture medium with actinomycin C1, 1 μg/ml for 3 hrs at 37℃ and 5% CO_2.
2. Seed cells at a concentration 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 concentration 0.001 - 0.5 ng/ml) in microtiter plates (tissue culture grade, 96 wells, flat bottom).
3. Incubate cell cultures for 24 hrs at 37℃ and 5% CO_2.
4. Add 10 μl/well Premix WST-1 and incubate for 4 hrs at 37℃ and 5% CO_2.
5. Measure the absorbance as described in the protocol (VI- 2).

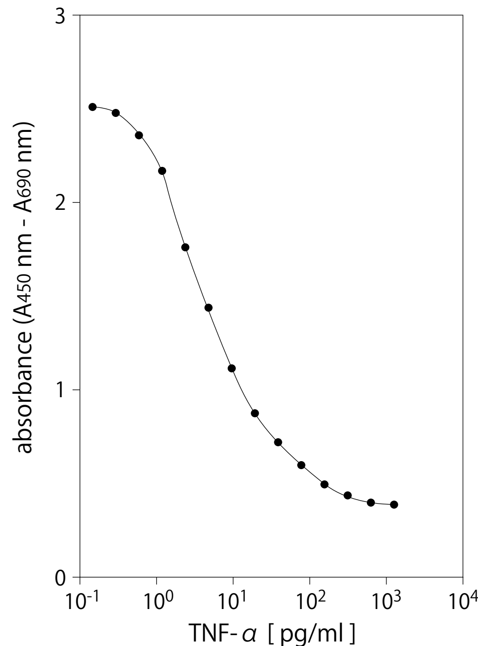

Figure 3. Measurement of the cytotoxicity of human TNF-α to WEHI-164 cells

VIII. Q & A

Q-1. What does Premix mean ?
A-1. With conventional WST-1 kits , the electron coupling reagent and WST-1 have to be mixed before the mesurement. This mixture could be stored only for 3 days at 4℃ or for one month at -20℃. Takara's Premix WST-1 is a ready-to-use solution in which all reagents are mixed. No need to mix before use. Futhermore, the Premix can be stored for at least one year at -20℃.
Even if once thawed, it is stable for at least 2 weeks if stored at 4℃. This kit is appropriate for customers who do not use this kit frequently.
Premix WST-1 allows more sensitive detection than conventional kits that have to be mixed before the mesurement.

Q-2. In measurement of cell proliferation, various tetrazolium salts (MTT, XTT, MTS, and so on) are used. How is Premix WST-1 different from these ?
A-2. Principle of the measurement is the same, but formazan dyes derived from each tetrazolium salt are completely different in water solubility. In the case of MTT, insoluble formazan dye crystals are formed, and the crystal need to be solubilized with surfactants or organic reagents. In the case of XTT and MTS, a soluble formazan dye is formed, but its solubility is lower than that of WST-1. WST-1 is the most sensitive and possesses the widest range compared with other tetrazolium salts.

Q-3. What is the difference between WST-1 and AlamarBlue, a water-soluble reagent for cell proliferation ?
A-3. Cell proliferation can be detected in absorbance and fluorescence with AlamarBlue. In case that the same sensitity as WST-1 is desired using AlamarBlue, cell proliferation has to be measured in fluorescence.

Q-4. Do components of medium or additives affect measurement ?
A-4. Theoretically, reducing substances may affect the value of absorbance. In the case that reducing reagents are added, cells should be washed with PBS once before measuring.

Q-5. How long does it take to preculture the cells before adding Premix WST-1 ?
A-5. The time for adding Premix WST-1 depends on the cell type, the cell phase (growth phase, static phase, etc.), and the objectives of the experiment. Usually overnight (16 hrs) is enough for preculture. In the "Experimental Example" section, all experiments were performed after 2 - 3 hr preculture.

Q-6. Is the measurement affected by the lactate dehydrogenase (LDH) derived from dead cells ?
A-6. The activity of LDH is often measured to detect the activity of cell injury. However, as LDH activity of dead cells is much lower than dehydrogenase activity of viable cells, the LDH activity does not affect the results of measurements.

Q-7. What wavelength is used for the measurement of formazan dye ?
A-7. A wavelength between 420 nm and 480 nm is appropriate. The Maximum absorbance is at 440 nm. The absorbance decreases when the wavelength is out of that range.

IX. Quick Protocol

Step	Procedure	Volume (μl well)	Time
1	Perform cell culture in 96-well microtiter plate.	100 μl/well 0.1 - 5.0 x 10^4 cells/well	24 - 96 hrs
2	Add Premix WST-1 and incubate at 37℃ in a humidified atmosphere.	10 μl/well	0.5 - 4 hrs
3	Measure absorbance using an ELISA reader at 420 nm, with a reference wavelength > 650 nm.		

X. References

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