WST-1 Cell Proliferation Assay Kit

RUO *Research Use Only* REF *15092* Σ 500 -25 ℃ -15 ℃

DESCRIPTION

- Cell proliferation assay is widely used in cell biology for the study of growth factors, cytokines or media components.
- WST-1, which is Tetrazolium salt, is especially useful for assaying the quantification of viable cells.
- WST-1 Assay Kit measures cell viability based on activity of mitochondria enzymes in live cells that reduce WST-1 and are inactivated shortly after cell death.
- The formazan dye formed in the assay is soluble in aqueous solution and can be quantified by measuring the absorbance at wavelength 440nm using a spectrophotometer.

INTRODUCTION

The WST-1 Cell Proliferation Assay Kit provides a fast and sensitive quantification method to measure cell proliferation and viability. The assay is based on the cleavage of the tetrazolium salt WST-1 to formazan by cellular mitochondrial dehydrogenases. Expansion of the number of viable cells result in an increase mitochondrial dehydrogenases activity, which leads to the increase in the amount of formazan dye formed. The soluble yellow formazan dye produced by viable cells can be quantified by spectrophotometer (ELISA reader) by measuring the absorbance at 440 nm and 650nm(reference wavelength). Efficient reduction of WST-1 requires an electron coupling reagent. This kit includes both WST-1 solution and Electro Connecting Solution(ECS) for a convenient and simple assay. This new method is non-radioactive, rapid and more sensitive than MTT, XTT assays. The entire assay can be performed in the same microtiter plate and does not require extra steps like washing, harvesting and cell solubilization.

KIT CONTENTS

Label	Contain
WST-1 Reagent	5 ml
Electro Connecting Solution(ECS)	500 µl

STORAGE AND STABILITY

- **Storage** : The components of the kit are stable at -20°C, should be protected from light until the expiration date printed on the label. It is recommended to prepare appropriate aliquots (1ml is sufficient for assay with one 96-well microtiter plate) to avoid repeated thawing and freezing.
- **Please store as follows** : Once thawed, store at +2 to +8°C, protected from light, for several weeks. However, please note that the solution may become viscous. If so, warm up the solution to 37°C for 2-10 min as described below.

  Note: If precipitates or turbidity are observed upon thawing, warm up the solution to 37°C for 2-10 min and agitate to dissolve the precipitates.

  Centrifugation is not recommended because the working concentration would decrease. After being dissolved, the WST-1 reagent can be used without any limitations.

APPLICATIONS

- Measurement of cell proliferation in response to growth factors, cytokines, mitogens, and nutrients, etc.
- Analysis of cytotoxic and cytostatic compounds such as anticancer drugs, toxic agents and other pharmaceuticals.
- Assessment of physiological mediators and antibodies that inhibit cell growth.

NOTICE BEFORE USE

WST-1 Cell Proliferation Assay Kit is intended for research use only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations. WST-1 Cell Proliferation Assay Kit is developed, designed, and sold for research purpose only. It is not intended to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals.

ADDITIONAL REQUIRED EQUIPMENT

- Spectrophotometer (ELISA reader)
- Cell Culture Plate (96well)

PROTOCOL

1. The cells(0.1 – 5 x 10^4/well) should be cultivated in a 96-well microtiter plate in a final volume of 100 µl/well growth medium in the absence or presence of various amounts of the factors to be tested. Incubate cells during 24 – 96 hours.
2. Defrost the WST-1 Reagent and the Electro Connecting Solution(ECS) immediately in a 37°C bath prior to use. Swirl gently until clear solution is obtained.
3. To prepare a sufficient WST-1 mixture for one plate (96 wells), add 100 µl Electro Connecting Solution(ECS) to 1 ml of WST-1 Reagent.

   Note: Depending on the number of test, mix Electro Connecting Solution(ECS) to WST-1 Reagent with proportion of 10:1
4. Add 10 µl of the WST-1 mixture to each well and incubate the plate in an incubator for 2 – 24 hours depending on cell density and the characteristics of the cell. (usually, 2 – 5 hours are sufficient).
5. Shake the plate gently to evenly distribute the dye in the wells.
6. Measure the absorbance of the samples against a background control as a blank with a spectrophotometer(ELISA reader) at a wavelength of 440 nm. In order to measure reference absorbance(to measure nonspecific readings), use a wavelength of 650 nm and subtract from the 440 nm measurement.

TROUBLE SHOOTING GUIDE

Problem / Possible cause	Recommendation
Low absorbance readings	• Prepare the WST-1 mixture immediately before use. • Increase incubation time with the WST-1 mixture. • Increase seeding density of cells. • Ensure the WST-1 Rreagent and Electro Connecting Solution are in solution before beginning the assay.
Poor replicates	• Ensure no bubbles are present in wells. • Pipette cells and/or WST-1 mixture accurately. • Check the accuracy of the pipette. • Ensure WST-1 Reagent and/or Electro Connecting Solution are fully dissolved before use.
High background	• Check proper storage of WST-1 at ≤ -20℃ in a manual defrost freezer. • Use freshly made WST-1 mixture. • Ensure media is free of microbial contamination. • Serum will contribute to reduction of WST-1. If possible, eliminate or reduce serum before adding WST-1 mixture.
Assay not working	• Assay buffer must not be chilled - needs to be stored at RT • Unsuitable microtiter plate for assay - Fluorescence: Black plates - Luminescence: White plates - Colorimetry: Clear plates

ORDERING INFORMATION

Product Name	Amount	Cat. No.
iN-fect™ *in vitro* Transfection Reagent	500 µl	15081
MTT Cell Proliferation Assay Kit	1000 assays	21180
XTT Cell Proliferation Assay Kit	500 assays	15091
i-poly Cell Culture Plate (96well)	50 ea/cs	IPY-31096

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