WST Plus-8, Cell Proliferation Reagent, Ready-to-Use

W0200

Storage

WST Plus-8 is exceedingly stable one year at 2-4°C.

For longer storage, store at -20°C for 24 months.

Repeated thawing and freezing will cause an increase in the background, which will interfere with your assay.

Please store at 2-4°C for frequent use.

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Introduction

WST Plus-8 is a great tool for monitoring cell viability. There are a variety of parameters that can be used for monitoring cell viability. WST Plus-8 uses water-soluble WST-8 tetrazolium salt to quantify the number of live cells. The water-soluble WST-8 tetrazolium salt produces a water-soluble orange formazan dye upon bioreduction in the presence of an electron carrier, 1-methoxy-5-methyl phenazinium methyl sulfate.

This reagent is convenient and robust with a mix and read format. WST Plus-8 reagent is added directly to the test cells, no pre-mixing of components are required. WST Plus-8 tetrazolium salt is reduced by cellular dehydrogenases to an orange formazan product that is soluble in tissue culture medium. The amount of formazan produced is directly proportional to the number of living cells by monitoring absorbance increase at 450 nm.

The excellent stability and little cytotoxicity of WST Plus-8 solution provide the benefits for the assays that require long incubation (such as 24 to 48 hours).

WST Plus-8 provides a sensitive colorimetric assay for the determination of the number of viable cells in the proliferation and cytotoxicity assays. The detection sensitivity is higher than any other tetrazolium salt-based assays such as MTT, XTT or MTS etc

Required Equipment and Materials

- Microplate reader (450 nm filter)
- 96-well plate
- CO_2 incubator
- 10 µl and 100 - 200 µl multi-channel pipettes

Cell Counting Assay

1. Inoculate cell suspension (100 µl/well) in a 96-well plate. Pre-incubate the plate in a humidified incubator (e.g., at 37°C, 5% CO_2).
2. Add 10 µl of the WST Plus-8 reagent to each well of the plate. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
3. Incubate the plate for 1 - 4 hours in the incubator.
4. Measure the absorbance at 450 nm using a microplate reader.

Note: To measure the absorbance later, add 10 µl of 1% w/v SDS or 0.1 M HCl to each well, cover the plate and store it with protection from light at room temperature. No absorbance change should be observed for 24 hours.

Cell Counting Assay

1. Dispense 100 µl cell suspension (about 5000 cells/well) in a 96-well plate. Pre-incubate the plate for 24 hours in a humidified incubator (at 37°C, 5% CO_2).
2. Add 10 µl of various concentrations of substances to be tested to the plate.
3. Incubate the plate for an appropriate length of time (e.g., 6, 12, 24 or 48 hours) in the incubator.
4. Add 10 µl of WST Plus-8 solution to each well of the plate. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
5. Incubate the plate for 1 - 4 hours in the incubator.
   Before reading the plate, you can mix gently on an orbital shaker for homogenization. Measure the absorbance at 450 nm using a microplate reader.

Note: To measure the absorbance later, add 10 µl of 1% w/v SDS or 0.1 M HCl to each well, cover the plate and store it with protection from light at room temperature. No absorbance change should be observed for 24 hours.

Creating a Standard Curve

1. Count the number of cells in the cell suspension using a hemocytometer or cell counter.
2. Proportionally dilute the cells with medium to a concentration gradient, generally 5-7 concentration gradient is necessary, several replicate wells per group is recommended. And then inoculate the cells. (Pay attention to the numbers of cells per well. If you dilute a cell suspension in a tube, please be careful to mix the cells to homogenize once again before adding to the well of the plate. The volume of the cell suspension in each well of the plate should be consistent.)
3. Incubate until cells adhering to the well (generally 2-4 hours), then add 10 µL of WST Plus-8 per 100µL medium. Continue incubating the plate for 1 - 4 hours, measure the absorbance at 450 nm using a microplate reader. Constructing the standard curve by plotting the number of cells on the X-axis and the absorbance on the Y-axis.

Note: The number of cells of the tested sample can be determined based on this curve. The prerequisite for using this standard curve is that the test conditions are identical.

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