

Cell Meter[™] Colorimetric WST-8 Cell Quantification Kit

Catalog number: 22770, 22771 Unit size: 1000 Tests, 5000 Tests

Component	Storage	Amount	
		Cat No. 22770	Cat No. 22771
WST-8 [™] Solution	Refrigerate (2-8 °C), Minimize light exposure	1 bottle (10 mL)	1 bottle (50 mL)

OVERVIEW

Our Cell Meter™ assay kits are a set of tools for monitoring cell viability. There are a variety of parameters that can be used for monitoring cell viability. Cell Meter™ Colorimetric WST-8 Cell Quantification Kit 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-methylphenazinium methyl sulfate. The kit is convenient and robust with a mix and read format. WST-8 solution is added directly to the test cells, no pre-mixing of components is required. WST-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 460 nm. The excellent stability and little cytotoxicity of WST-8 solution make the kit useful for the assays that require long incubation (such as 24 to 48 hours). Cell Meter™ Colorimetric WST-8 Cell Quantification Kit 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.

AT A GLANCE

Protocol summary

- 1. Prepare cells in a 96-well plate (100 µL/well)
- 2. Add 10 µL of WST-8[™] Solution to each well
- 3. Incubate at 37°C for 1 4 hours
- 4. Monitor absorbance at OD = 460 nm

Important WST-8TM Solution is stable for more than one year if store at 4°C and protected from light. Store it at <-20°C for longer storage.

KEY PARAMETERS

Instrument: Absorbance: Recommended plate: Absorbance microplate reader 460 nm Clear bottom

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit <u>https://www.aatbio.com/resources/guides/cell-sample-preparation.html</u>

SAMPLE EXPERIMENTAL PROTOCOL

Cell Proliferation and Cytotoxicity Assay:

- 1. Plate 5000 to 10,000 cells/well in a tissue culture microplate with clear bottom.
- 2. Add test compounds into the cells and incubate for a desired period of time (such as 24, 48 or 96 hours) in a 37°C, 5% CO₂ incubator. For blank wells (medium without the cells), add the same amount of test compounds. The suggested total volume is 100 μL for a 96-well plate, and 50 μL for a 384-well plate.
 - *Note* Each cell line should be evaluated on an individual basis to determine

the optimal cell density for proliferation or cytotoxicity induction. For proliferation assays, use fewer cells; for cytotoxicity assays, use more cells to start with.

- Add 10 μL/well (96-well plate) or 5 μL/well (384-well plate) of WST-8[™] Solution to each well.
- 4. Incubate the plate at 37°C for 1 4 hours, protect from light.

Note The incubation time could be from 30 minutes to overnight depending on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

5. Monitor the absorbance increase with an absorbance plate reader at OD =460 $\,$ nm.

Cell Counting Assay:

1. Prepare cell culture in a tissue culture microplate with clear bottom. The suggested total volume is 100 μL for a 96-well plate or 50 μL for a 384-well plate.

Note We used serially diluted HeLa and Jurkat cell suspension in a clear bottom 96-well plate for the assay.

- 2. Add 10 μL /well (96-well plate) or 5 μL /well (384-well plate) of WST-8^TM Solution to each well.
- 3. Incubate the plate at 37°C for 1 4 hours, protect from light.

Note The incubation time could be from 30 minutes to overnight depending on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

 Monitor the absorbance increase with an absorbance plate reader at OD = 460 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (Abs (460 nm)) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Number of Cells/ well samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-onlinecalculator



Figure 1. Cell number was determined with Cell Meter[™] Colorimetric WST-8 Cell Quantification Kit. HeLa cells at 0 to 10,000 cells/well/100 μL were added in a clear bottom 96-well plate. The absorbance was measured at 460 nm using a SpectraMax reader (Molecular Devices).

DISCLAIMER

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