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07553E_1908_8

Product No. 07553

Cell Count Reagent SF

Cell Count Reagent SF allows sensitive colorimetric assays by utilizing highly water-soluble tetrazolium salt.
WST-8[2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4 -disulfophenyl)-2H-tetrazolium, monosodium salt]* produces a water-soluble formazan dye upon reduction in the presence of an electron carrier as shown in Figure 1 .
Since the absorbance at 450 nm is proportional to the number of viable cells in the medium, the viable cell number can be determined using the absorbance value of a previously prepared calibration curve.
This product is a one-bottle solution; no premixing of components is required.

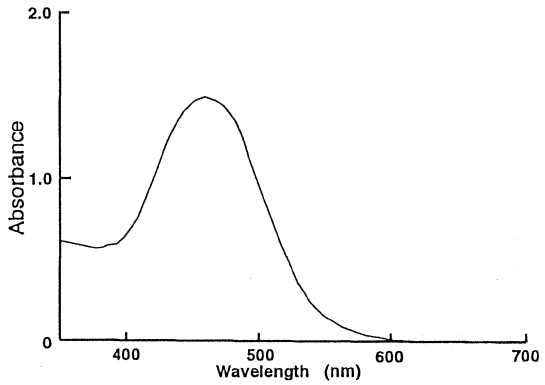

Figure 1: absorption spectrum of WST-8 formazan

Features

- No radioisotope
- No solubilization steps for formazan
- More sensitive than other water-soluble tetrazolium salt (XTT, MTS)
- Ready to use
- More stable than other kits

Components

Mixed solution of WST-8 and 1-Methoxy PMS.

Stability

- Store at refrigerator with protection from light, for frequently use.
- Store at freezer with protection from light, for extended period.
- Thaw it leaving at room temperature or in warm water(less than 37°C). Avoid repeated thawing and freezing.

Protocol

Cell Proliferation Assay

1. Prepare a cell suspension using an appropriate culture medium, and dispense 100µl of cell suspension into each wells of a 96-well plate after counting cells*1.
2. Pre-incubate the medium in CO_2 incubator.
3. Add 10µl of Cell Count Reagent SF to each wells of the plate*2.
4. Incubate the medium for 1-4 hours in the incubator*3.
5. Measure the absorbance at 450 nm (Wave length: 600nm or more) by micro plate reader*4.

*1 Usable the medium with phenol red.
*2 Sterilize the solution by filter with 0.22µm membrane, as necessary.
*3 Consider the condition. The absorbance is different among the type or number of cells.How to stop the reaction.
1) Cool down the plate to 4°C.
2) Add 10µl of 0.1mol/l HCl.
3) Add 10µl of 1w/v%SDS. Measure the absorbance within 24 hours.

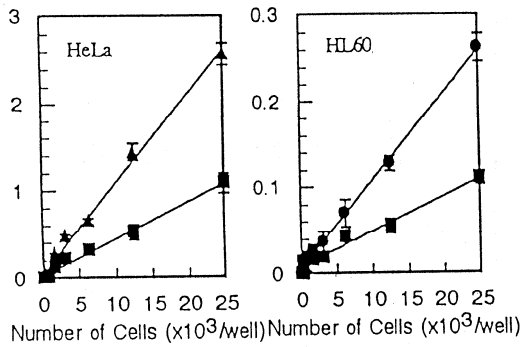

Figure 2: Cell proliferation assay

*4 Use 430 -490nm filter. Wavelength of maximum absorbance for formazan is around 460nm.

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Cytotoxicity Assay

1. Prepare a cell suspension with 5,000 cells/well using an appropriate culture medium. Add 100 µl of the cell suspension to each well of a 96-well plate.
2. Pre-incubate the medium in CO_2 incubator for 24 hours.
3. Add 10µl of the concentration prepared toxicant on each well of the plate.
4. Incubate the medium for 48 hours in CO_2 incubator.
5. Add 10µl of Cell Count Reagent SF to each well of the plate.
6. Incubate the medium for 1-4 hours in CO_2 incubator.
7. Measure the absorbance at 450 nm (Wave length: 600nm or more) by microplate reader.

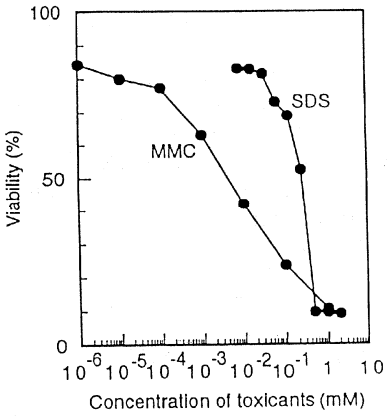

Figure 3: Toxicological test of chemicals

Storage

Refrigerator, Protect from light

Packing

500TESTS (Product No:07553-15)
2500TESTS (Product No:07553-44)

Reference

M. Ishiyama, Y. Miyazono, K. Sasamoto, Y. Ohkura, K. Ueno, *Talanta* **44**, 1299(1997)
H. Tominaga, M. Ishiyama, F. Ohseto, K. Sasamoto, T. Hamamoto, K. Suzuki and M. Watanabe, *Anal. Commun*, **36(2)**, 47(1999)

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