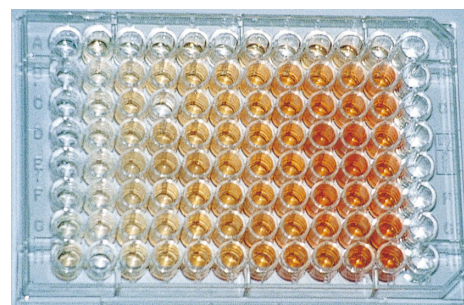


Cell Count Reagent SF

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.

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.

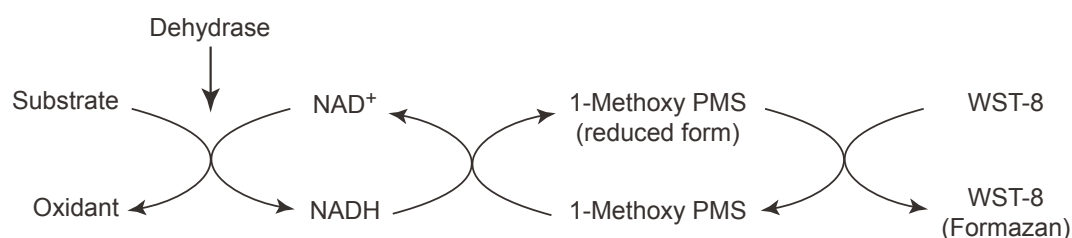
This product is a one-bottle solution; no premixing of components is required.



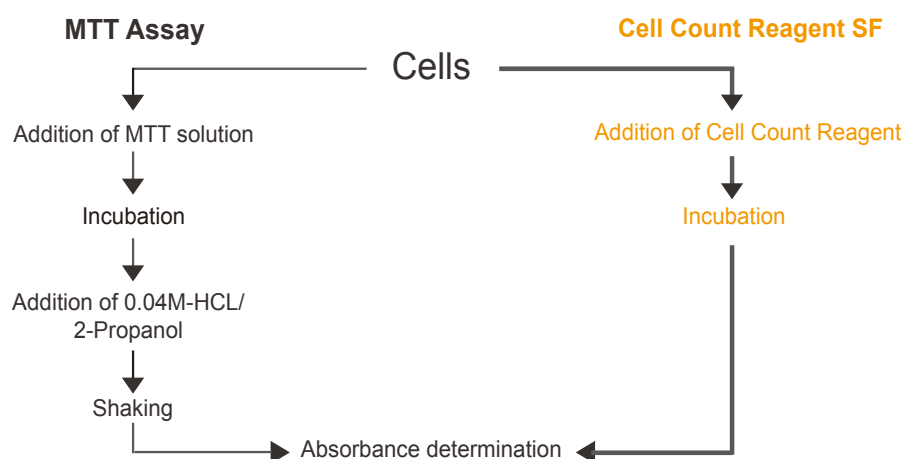
Features

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

Principle

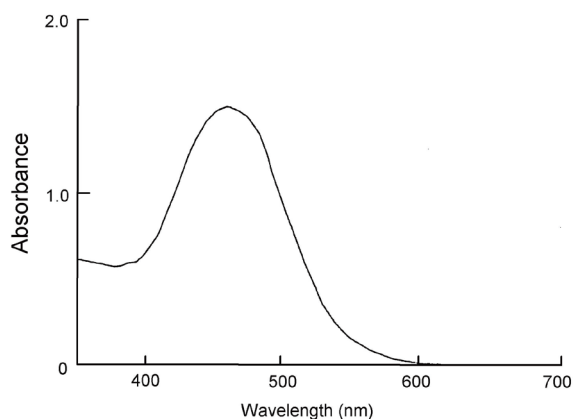


Comparison of Assay Procedure with MTT and Cell Count Reagent SF



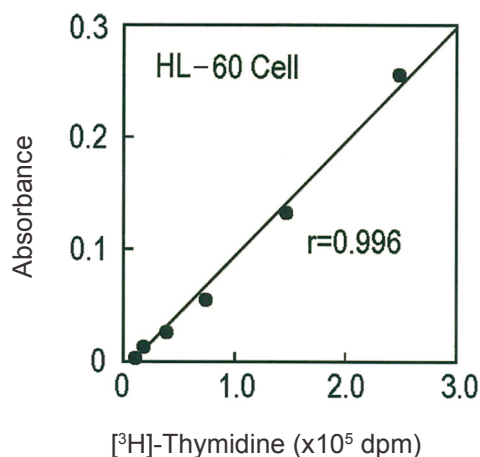
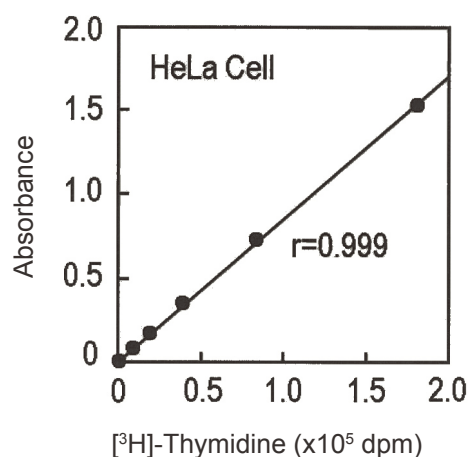
Absorption Spectrum

Absorption spectrum of WST-8 formazan



Correlation with [³H]-Thymidine

Absorbance vs. [³H]-Thymidine



Application for Cell Proliferation Assay

1. Prepare a cell suspension using an appropriate culture medium, count cells to the desired number and dispense 100 μ l of suspension of cells in logarithmic growth phase into each well of a 96-well plate^{*1}.
2. Pre-incubate the medium in CO₂ incubator.
3. Add 10 μ l of Cell Count Reagent SF to each well of the plate^{*2}.
4. Incubate the medium for 1-4 hours in the incubator^{*3}.
5. Measure the absorbance at 450 nm (calibration wavelength: 600 nm or higher) by micro plate reader^{*4}.

*1 Usable with media containing phenol red.

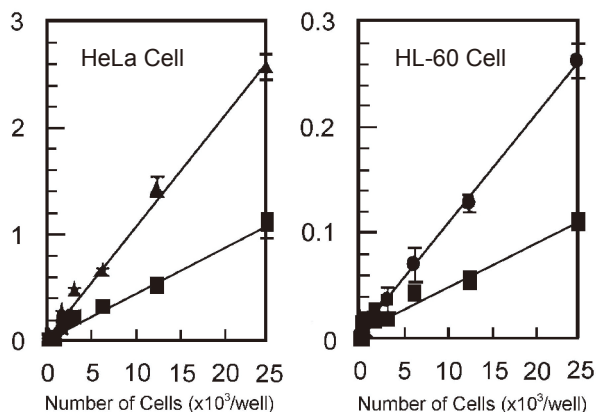
*2 Sterilize the solution by filtration with 0.22 μ m membrane, as necessary.

*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 10 μ l of 0.1mol/l HCl.
- 3) Add 10 μ l of 1w/v% SDS. Measure the absorbance within 24 hours.

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

Application for Cell Proliferation Assay (Continued)



Media

HeLa: DMEM1640 (10% FBS)

HL-60: RPMI1640 (10% FBS)

Incubation

HeLa cells: 5% CO₂, 37°C, 1 hour (■), 2 hours (▲)

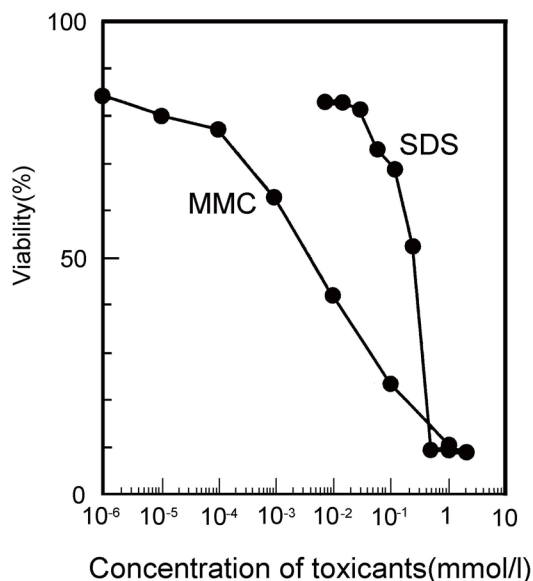
HL-60: 5% CO₂, 37°C, 1 hour (■), 3 hours (●)

Measurement Wavelength

450 nm (calibration wavelength: 650 nm)

Cytotoxicity Assay

1. Prepare a suspension of cells in logarithmic growth phase 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 CO₂ incubator for 24 hours.
3. Add 10 μl of the prepared toxicant on each well of the plate.
4. Incubate the medium for 48 hours in CO₂ incubator.
5. Add 10 μl of Cell Count Reagent SF to each well of the plate.
6. Incubate the medium for 1-4 hours in CO₂ incubator.
7. Measure the absorbance at 450 nm (calibration wavelength: 600 nm or higher) by microplate reader.



Cell: HeLa

Media: RPMI1640 (10% FBS)

Toxicants: Mitomycin C (MMC)
Sodium Dodecylsulfate (SDS)

Pre-Incubation: 37°C, 5% CO₂, 48 hours

Incubation: 37°C, 5% CO₂, 2 hours

Measurement Wavelength:
450 nm (calibration wavelength: 650 nm)

References

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)

Storage

Keep refrigerated, and protect from light.

For long-term storage, keep at -20°C and protect from light.

To thaw, leave at room temperature, or place in water 37°C or cooler.

Avoid repeated freeze-thaw cycles.

Packing

500TESTS : contains 5 ml x 1 vial (Product No:07553-15)

2500TESTS: contains 5 ml x 5 vials (Product No:07553-44)

This reagent is a solution containing WST-8 and 1-methoxy PMS.

One vial contains enough material for 500 tests, or five 96-well microtiter plates.

Ordering Information

Product Name	Storage	Product No.	PKG Size
Cell Count Reagent SF	-20°C	07553-15	500 tests
		07553-44	2500 tests

Related Product

Product Name	Storage	Product No.	PKG Size
MTT Cell Count Kit	-20°C	23506-80	1 Kit

Components

MTT solution (5 mg/ml MTT/PBS) 2 ml x 5

Solubilization solution (Isopropanol with 0.04 mol/l HCl)

1 kit for 1,000 assays

For research use only, not intended for diagnostic or drug use.

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