ZETA™
L I F E

DATASHEET

Cell Counting Kit

Cell Proliferation Assay - Cytotoxicity Assay

General Information

Cell Counting Kit(CCK) allows very convenient assays by utilizing ZETA LIFE's highly water-soluble tetrazolium salt.WST-8[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 mediator, as shown in Figure1.

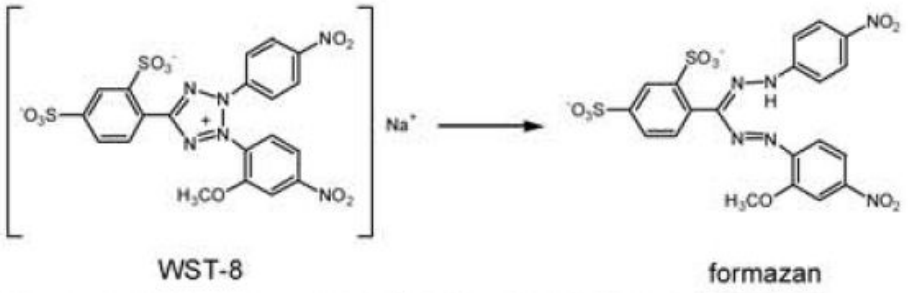

Figure 1. Structures of WST-8 and WST-8 formazan

Figure 1.Structures of WST-8 and WST-8 formazan

CCK is a one-bottle solution;no premixing of components is required.CCK ,being nonradioactive,allows sensitive colorimetric assays for the determination of the number of viable cells in cell proliferation and cytotoxicity assays.WST-8 is reduced by dehydrogenases in cells to give an orange colored product(formazan),which is soluble in the tissue culture medium(figure2).

the amount of the formazan dye generated by dehyhrogenases in cells is directly proportional to the number of living cells.

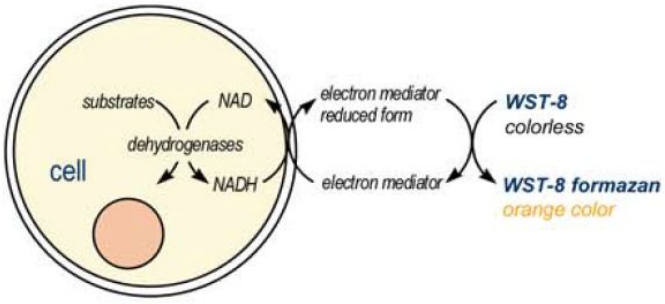

Figure 2. Principle of the cell viability detection with Cell Counting Kit.

Figure3 shows that the cell proliferation assay using CCK correlates well with the [^{3}H]-thymidine incorporation assay. Thus,the CCK assay can also be substituted for the [^{3}H]-thymidine incorporation assay.As is higher than that using other tetrazolium salts such as MTT,XTT,MTS or WST-1.

50tests :	0.5ml×1tube
100tests :	1 ml ×1tube
500tests :	5 ml ×1bottle
1000tests :	10 ml×1bottle
3000tests :	30 ml×1bottle

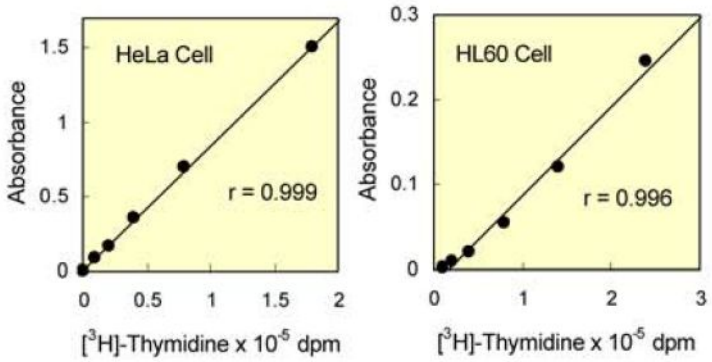

Figure 3. Correlation between CCK assay and [^{3}H]-thymidine incorporation assay.

Medium : HeLa.....MEM, 10% FBS
HL60.....RPMI1640, 10% FBS
Reagent : [^{3}H]-Thymidine.....37 KBq/well
CCK.....10 µl/well
Incubation : [^{3}H]-Thymidine assay..... 4 hours
CCK.....3 hours

CCK is stable over one year at 4℃ with protection from light .Store it at -20℃ for longer storage . Repeated thawing and freezing causes an in crease in the background, which interferes with the assay .Please store the kit at 4 ℃ for frequent use.

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

Number of Cells Determination

1.Inoculate cell suspension (100ul/well） in a 96-well plate.Pre-incubate the plate in a humidified incubator(e.g.,at 37℃， 5% CO_2).
2.Add 10ul of the CCK solution to each well of the plate.
Be careful not to introduce bubbles to the wells ,since they interfere with the O.D.

Reading.

4. Incubate the plate for 1 - 4 hours in the incubator.

5. Measure the absorbance at 450 nm using a microplate reader.

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 .

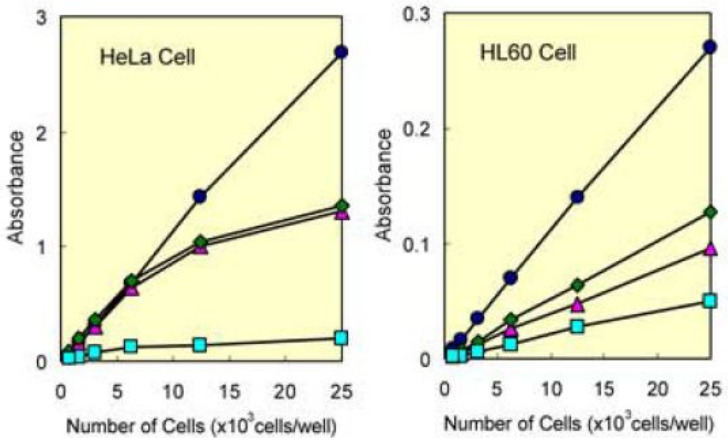

Figure 4. Number of cells determination using CCK and other reagents.

Medium :	*HeLa..... MEM, 10% FBS*	
	HL60..... RPMI1640, 10% FBS	
Incubation :	*HeLa.....37 °C, 5 % CO_2, 2 hours*	
	HL60.....37 °C, 5 % CO_2, 3 hours	
Detection :	*CCK (●) ... 450 nm,*	*XTT (◆)....450 nm*
	MTS (▲)..... 490 nm,	*MTT (□)... 570 nm*

Cell Proliferation and Cytotoxicity Assay

1.Dispense 100 µl of cell suspension (5000 cells/well) in a 96-well plate. Pre-incubate the plate for 24 hours in a humidified incubator (e.g., 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 CCK 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.

6. Measure the absorbance at 450 nm using a microplate reader.

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.