INSTRUCTIONS

# Cell Counting Kit – 8

**Package Contents:** 1 vial

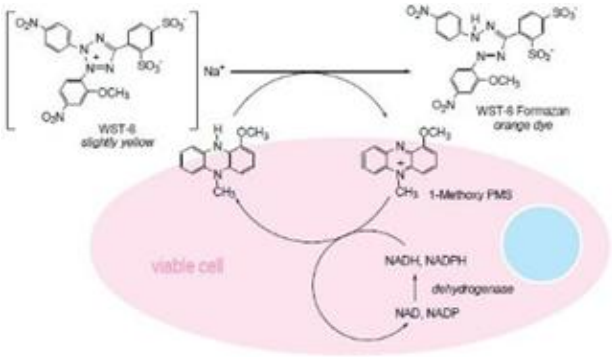
| Cell Counting Kit – 8 | | | | | |
|---|---|---|---|---|---|
| Catalog No. | Package | Price | Quantity/Unit | Form | Sipping and Storage Guidelines |
| R-CK100 | 100 T | 50 | 1 bottle | Liquid. Light to deep red. | Stable for 2 years at -20 °C, 1 year at 4 °C and 6 months at room temperature with protection from light. To avoid repeated thawing and freezing, keep the kit at 4 °C for frequent use. |
| R-CK500 | 500 T | 200 | | | |
| R-CK1000 | 1000 T | 360 | | | |
| R-CK3000 | 3000 T | 1000 | | | |

## Shipping and Storage

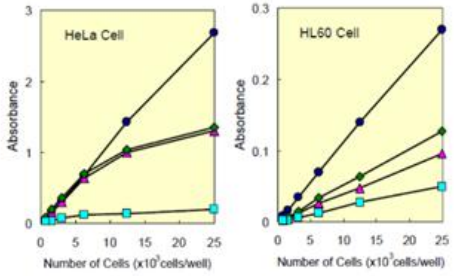
Stable for 2 years at -20 °C, 1 year at 4 °C and 6 months at room temperature with protection from light. To avoid repeated thawing and freezing, keep the kit at 4 °C for frequent use.

## DESCRIPTION

Cell Counting Kit-8 (CCK-8) provides a very convenient assay by using tetrazolium salt WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt), which can be reduced into a water-soluble formazan dye in the presence of an electron carrier, 1-Methoxy PMS.



While using this kit, CCK-8 solution can be added directly to cells and no pre-mixing of components is needed. Then WST-8 in the solution is reduced by cellular dehydrogenases to the orange formazan, which is soluble in tissue culture medium. The amount of produced formazan is directly proportional to the number of living cells. The stability and little cytotoxicity of WST-8 allow this kit useful for assays that need long incubation (such as 24 to 48 hours).




Cell Counting Kit-8 allows colorimetric assays to detect the viable cells number in the proliferation and cytotoxicity experiments. This detection assay is more sensitive than any other tetrazolium salts such as MTT, XTT or MTS.

## ADVANTAGES

1. One-bottle, ready-to-use solution.

2. No organic solvents or isotopes required.

3. No harvesting, no washing and no solubilization steps.

4. More sensitive than MTT, XTT, MTS or WST-1.

## PROCEDURE

**Required Equipment and Materials:**

- plate reader (450 nm filter)
- 96-well plate
- CO2 incubator
- 10µl, 200µl and multi-channel pipettes

**Cell Proliferation Assay:**

1. Inoculate cell suspension (about 100µl/well) in a 96-well plate. Pre-incubate the plate in a humidified incubator (e.g., at 37°C, 5% CO2).

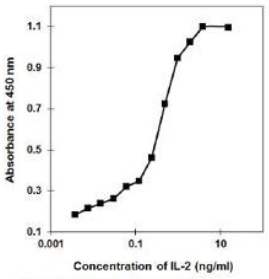
2. Add 10µl of the CCK-8 solution to each well of the plate.

***Caution:***

*Avoid introducing bubbles to the wells, since they interfere with the O.D. reading.*

3. Incubate the plate for 1 to 4 hours in the incubator.

4. Measure the absorbance at 450 nm using a microplate reader. Prepare a calibration curve using the data obtained from the wells that contain known numbers of viable cells.




**Response by Cytokine**
**Cell** : CTLL-2 Cells
**Medium**: RPMI1640 with FBS
**Drug** : Human Interleukin-2
**Exposure** : 37°C, 5% $CO_2$, 72 hr

***Note:***

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

**Cytotoxicity Assay:**

1. Dispense 100 µl of cell suspension (5000 cells/ well) in a 96-well plate.

2. Pre-incubate the plate for 24 hours in a humidified incubator.

3. Add 10 µl of various concentrations of toxicant into the culture media.

4. Incubate the plate for appropriate time in the incubator.

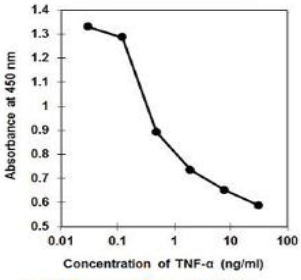
5. Thaw the CCK-8 on the bench top or in a water bath at 37 °C if it is frozen.

***Note:***
*It takes about 30 minutes on the bench top at 25 °C while 5 minutes in the 37 °C water bath.*

6. Add 10µl of CCK-8 solution to each well of the plate.

***Caution:***
*Avoid introducing bubbles to the wells, since they interfere with the O.D. reading.*

7. Incubate the plate for 1-4 hours in the incubator. Measure the absorbance at 450 nm with a microplate reader.




**Anti Cancer Drug Activity**
**Cell** : HeLa Cells
**Medium**: DMEM with FBS
**Drug** : TNF-α with 1 mg/ml Actinomycin D
**Exposure** : 37°C, 5% CO , 5 hr

***Note:***

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

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## PRECAUTIONS

1. CCK-8 assay is based on the dehydrogenase activity detection in viable cells, thus conditions or chemicals that affect dehydrogenase activity may cause discrepancy between the actual and detected viable cell number.

2. WST-8 may react with reducing agents and generate formazan. Please check the background O.D. if reducing agents are used in cytotoxicity or cell proliferation assays.

3. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.

4. If sterilization is required, please filter the CCK-8 solution with a 0.22 μm filter.

5. The incubation time varies by the cell type and cell number in wells. Generally, leukocytes give weak coloration, thus a long incubation time (up to 4 hours) or a large number of cells (~105 cells/well) may be necessary.

6. Measure and subtract the O.D. at 600 nm or higher from that of sample if there is a high turbidity in the cell suspension.

## Contact Information

Genemedi Biotech. Inc.

For more information about reagents, please visit: https://www.genemedi.net/i/reagent
For more information about Genemedi products and to download manuals in PDF format, please visit our web site: www.genemedi.net
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