Cell Counting Kit

Cat #: orb90465 (manual)

Cell Counting Kit-8, being nonradioactive, allows sensitive colorimetric assays for the determination of the number of viable cells in cell proliferation cytotoxicity assays.

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

Product Overview

Form	Liquid
Size	5 mL (for 500 assays, 10µL per well)
Concentration	6mg/ml
Buffer	9% NaCl
Storage	Upon receipt store at -4°C, protect from light. Note: Repeated thawing and freezing causes an increase in the background, which interferes with the assay.
Stability	When stored as directed, product should be stable for one year. Store it at -20˚C for longer storage
Sensitivity	CCK-8 is the highest sensitive dye for the cell-based assay.

Introduction

Cell Counting Kit-8 (CCK-8) allows very convenient 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. Cell Counting Kit-8 is a one-bottle solution; no premixing of components is required. Cell Counting Kit-8, being nonradioactive, allows sensitive colorimetric assays for the determination of the number of viable cells in cell proliferation 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. The amount of the formazan dye generated by the activity of dehydrogenases in cells is directly proportional to the number of living cells. The cell proliferation assay using CCK-8 correlates well with the [(3)H]thymidine incorporation assay. And the CCK-8 assay can also be substituted for the [(3)H]thymidine incorporation assay. The detection sensitivity of CCK-8 is higher than other tetrazolium salts such as MTT, XTT, MTS or WST-1.

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. No Toxicity to Cell.

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

Precautions

1. Run pilot test to determine the optimal cell number and incubation time.
2. Since the CCK-8 assay is based on the dehydrogenase activity detection in viable cells, conditions or chemicals that affect dehydrogenase activity in viable cells may cause discrepancy between the actual viable cell number and the cell number determined using the CCK-8 assay.
3. WST-8 may react with reducing agents to generate WST-8 formazan. Please check the background O.D. if reducing agents are used in cytotoxicity assays or cell proliferation assays.
4. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
5. Phenol red containing culture media can be used with this kit for cell viability assays.
6. Membrane filtration is recommended for the sterilization of the CCK-8 solution, if necessary.
7. The incubation time varies by the type and number of cells in a well. 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.
8. Since the cytotoxicity of this kit is very low, further color development is possible after reading the absorbance.
9. Neutral red or crystal violet can be used after the CCK-8 assay.
10. Measure the reference wavelength at 600 nm or higher if there is a high turbidity in the cell suspension.

Additional Materials Required

10µL and 100-200µL Multichannel Pipettes Plate reader (450 nm filter)

96-well plate CO_2 incubator

General Protocol

Cell Number Determination

1. Inoculate cell suspension (100µL/well) in a 96-well plate. Pre-incubate the plate in a humidified incubator (e.g., at 37 ˚C, 5% CO_2).

2. Add 10µL of the CCK-8 solution to each well of the plate. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.

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

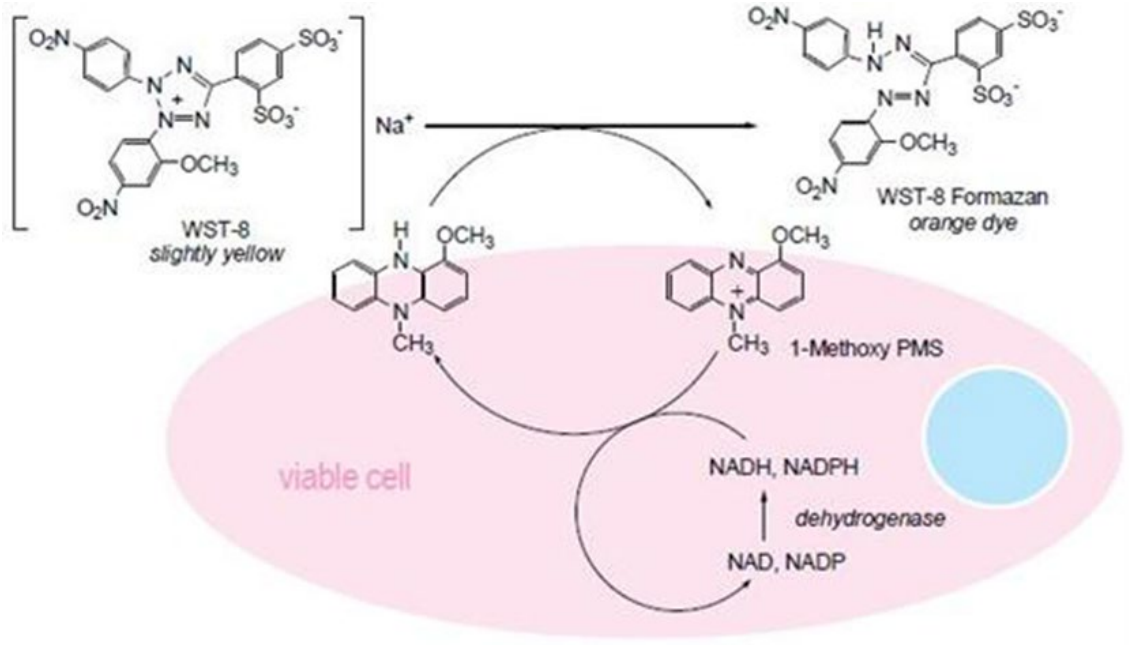
4. Measure the absorbance at 450 nm using a microplate reader. To measure the absorbance later, add 10µL of 1% w/v SDS or 0.1M HCl to each well, cover the plate and store it with protection from light at room temperature. No absorbance change should be observed for 24 hours.

Cell proliferation and Cytotoxicity Assay

1. Dispense 100µL 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 10µL 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 10µL of CCK-8 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 10µL of 1% w/v SDS or 0.1M 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.

Cell viability detection mechanism with CCK-8



Example Data using Cell Counting Kit-8

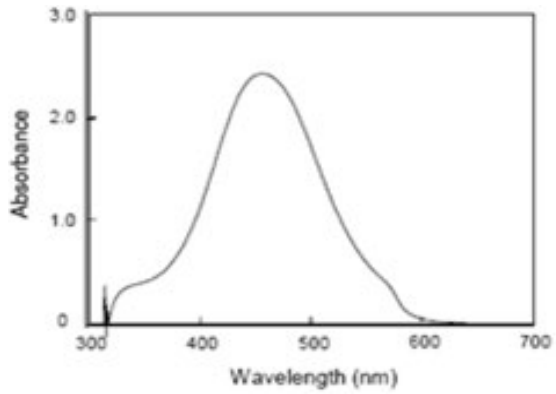


Figure 1. Absorption spectrum of WST-8 formazan

Figure 1 shows the absorption spectrum of WST-8 formazan. Since the absorbance at 460 nm is proportional to the number of viable cells in the medium, the viable cell number can be determined using the absorbance of a previously prepared calibration curve.

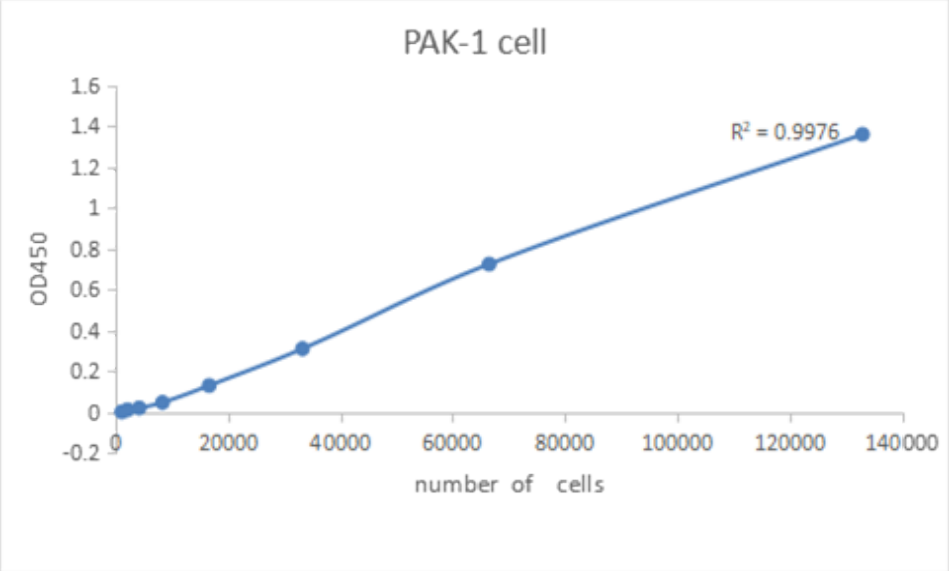


Figure 2. Cell proliferation assay using CCK-8

Culture medium: MEM, 10% FBS

Incubation: 37 ˚C, 5% CO2, 2 hours

Deteactin: 450nm

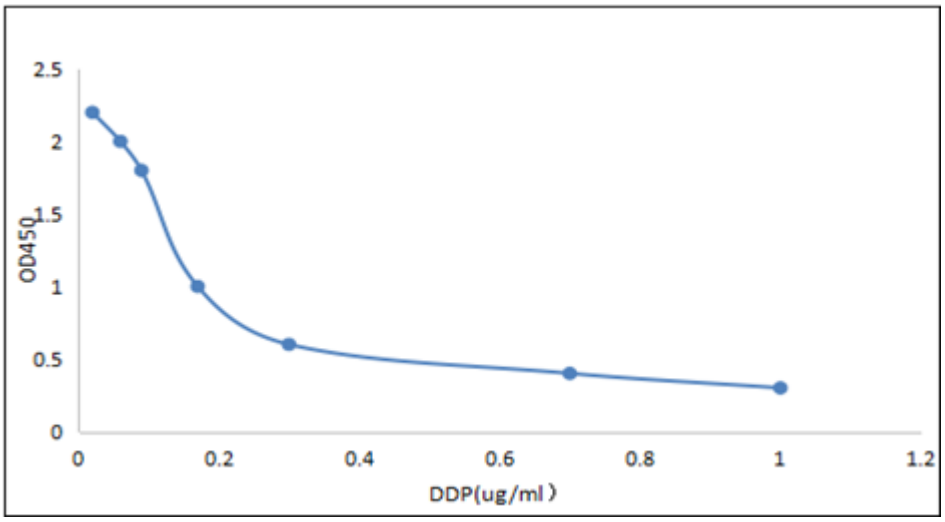


Figure 3. Toxicological test of chemicals using CCK-8

Cell line: hela

Medium: DMEM, 10% FBS

Chemicals: 200 μM Cisplatin (DDP) Incubation: 37°C, 5% CO_2, 2 hours

Frequently Asked Questions

1. How many cells should there be in a well?

For adhesive cells, at least 1000 cells are necessary per well (100µL medium). For leukocytes, at least 2500 cells are necessary per well (100µL medium) because of low sensitivity. The recommended maximum number of cells per well for the 96-well plate is 25000. If a 24 well or 6 well plate is used for this assay, please calculate the number of cells per well accordingly, and adjust the volume of the CCK-8 solution in a well to 10% of the total volume.

2. Does CCK-8 stain viable cells?

No. Since WST-8 and its formazan dye are highly water-soluble, CCK-8 cannot be utilized for cell staining purpose.

3. Does phenol red affect the assay?

No. The absorption value of phenol red in a culture medium can be removed by subtracting the absorption value of a blank solution from the absorption value of each well. Therefore, a medium containing phenol red is usable for the CCK-8 assay.

4. Is CCK-8 toxic to cells?

Since the toxicity of CCK-8 is so low, the same cells can be used for other cell proliferation assays such as the crystal violet assay, neutral red assay or DNA fluorometric assay after the CCK-8 assay is completed.

5. I do not have a 450 nm filter. What other filters can I use?

You can use filters with the absorbance between 430 and 490 nm, even though 450 nm filter gives the best sensitivity.