Enhanced Cell Counting Kit-8

Cat. No. **BA00208**

www.biossusa.com
support@biossusa.com
800.501.7654[DOMESTIC]
+1.781.569.5821[INT'L]

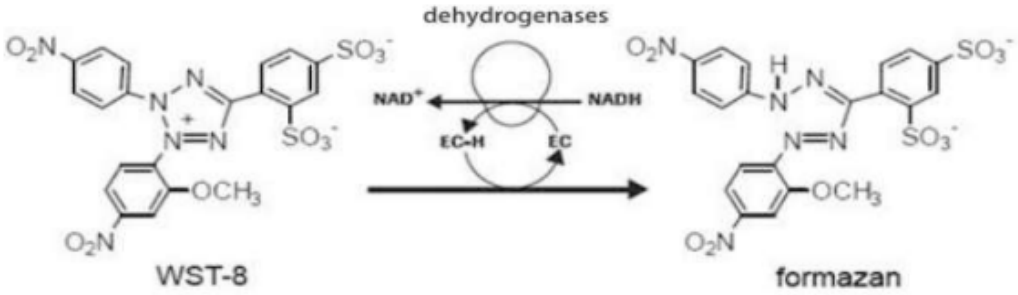
Background

General Information

Enhanced Cell Counting Kit- 8 (CCK-8) allows very convenient assays by utilizing the highly water-soluble tetrazolium salt WST-8.

WST-8 produces a water-soluble formazan dye upon reduction in the presence of an electron carrier and is reduced by dehydrogenases in cells to give a yellow-colored product namely formazan(as shown below).

The kit allows sensitive colorimetric assays for the determination of the number of viable cells in cell proliferation and cytotoxicity assays. The amount of the formazan dye generated by the activity of dehydrogenases in cells is directly proportional to the number of living cells.



Advantages:

- Low toxicity to cells
- One-bottle, no premixing of components
- No organic solvents or isotopes required
- No harvesting, no washing, and no solubilization steps
- More sensitive than MTT, XTT, MTS, or WST-1

Formulation and Storage

Storage

Store the kit at 4°C with protection from light for one year.
Store the kit at -20°C with protection from light for two years.

Kit Contents

:1ml(100T) / 5ml(500T) / 6*5ml(3000T)

General Protocol:

Cell Number Determination

1. Collect the cells needed for the experiment and then prepare wells that contain known numbers of viable cells.
2. It is recommended to preincubate adherent cells. After that add 10 μl of the CCK-8 solution to each well of the plate.
3. Incubate the plate for 1-4 hrs 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.
5. Under the completely consistent condition, the cell number of the unknown sample can be calculated according to the standard curve.

Cell Viability Assay

1. Inoculate cell suspension (above 5000 cells/100 μl/well) in a 96-well plate. The cells for the assay should enter into the logarithmic growth phase. The average incubation time to enter into this phase is from 24 hrs to 48 hrs. Please check cell databases to estimate the preincubation time.
2. Add 10μl of the CCK-8 solution to each well of the plate. Be careful not to introduce bubbles to the wells.
3. Incubate the plate for 1-4 hrs in the incubator. However, the absorbance will differ between cell types even if the number of cells/well and coloration time are the same. Set an appropriate incubation time to give a proportional relationship between the cell number and the absorbance.
4. Measure the absorbance at 450 nm using a microplate reader. To measure the absorbance later, add 10μl of 1% w/v SDS or 0.1 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 hrs.

Cell Proliferation and Cytotoxicity Assay

1. Dispense 100μl of cell suspension in a 96-well plate at a density of 5*103-105 cells/well. Pre-incubate the plate overnight or 24hrs in a humidified incubator.
2. Add various concentrations of the toxicant into the culture media in the plate.
3. Incubate the plate for an appropriate length of time (e.g., 3, 6, 12, 24, or 48 hrs) in the incubator.
4. Add 10 μl of CCK-8 solution to each well of the plate. Be careful not to introduce bubbles to the wells.
5. Incubate the plate for 1-4 hrs in the incubator. The incubation time varies by the type and number of cells in a well.
6. Measure the absorbance at 450 nm using a microplate reader. To measure the absorbance later, add 10μL of 1% w/v SDS 0.1 M HCl to each well, cover the plate and store it with protection from light at room temperature. Be sure to take a reading within 24 hrs after stopping the reaction.

The Formula to Calculate:

The ratio of cell viability = [(ODt-ODb) / (ODc-ODb)] × 100%

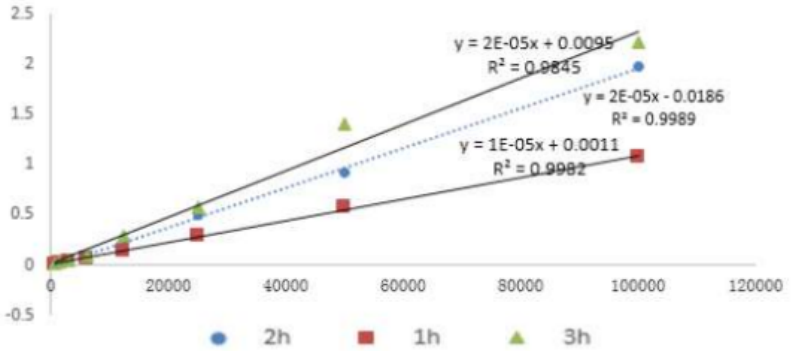
The ratio of inhibition = [(ODc-ODt) / (ODc-ODb)] × 100%

ODt: The absorbance of the test group (with cell、culture Medium、CCK-8 and toxicant)

ODc: The absorbance of the control (with cell、culture Medium and CCK-8, without toxicant)

ODb: The absorbance of blank (with culture medium and CCK-8, without cell and toxicant)

Quality verification:



Cell line:SP2/0、0-105/100ul

Culture Medium:DMEM,10%FBS;37°C,5% CO2

Incubation:1h\2h\3h

Precautions:

1. The number of cells in a well varies by the size of the cell, proliferation rate, and specific requirements of the experiment. The total number of cells is recommended to be 0-105 per well.

2. Prepare the well for background measurement which contains all materials except for cells. Measure the background absorbance of the well at 450 nm, and then subtract the background absorbance from the absorbance of the sample well containing all materials and cells.

3. Please add Cell Counting Kit-8 solution equal to 1/10 the volume of the media (if the media is 100μl, add 10μl of solution).

4. If the cell culture time is long and the medium color changes, remove the culture medium from cells and add new culture medium prior to adding CCK-8.

5. In order to fully mix the cck-8 and reduce the residue of cck-8 on the pipette, it is recommended to dilute the cck-8 reagent with culture medium before sample addition.

6. WST-8 may react with reducing agents to generate WST-8 formazan and give a false reading. Please check the background O.D. if reducing agents are used in cytotoxicity assays or cell proliferation assays. The absorbance from such dyes can be subtracted as a blank.

7. This product is for R&D use only, not for drug, household, or other uses.

8. For your safety and health, please wear lab clothes and disposable gloves.