Cell Counting Kit-8

User Manual

Catalog # CAK2007

(Version 1.1A)

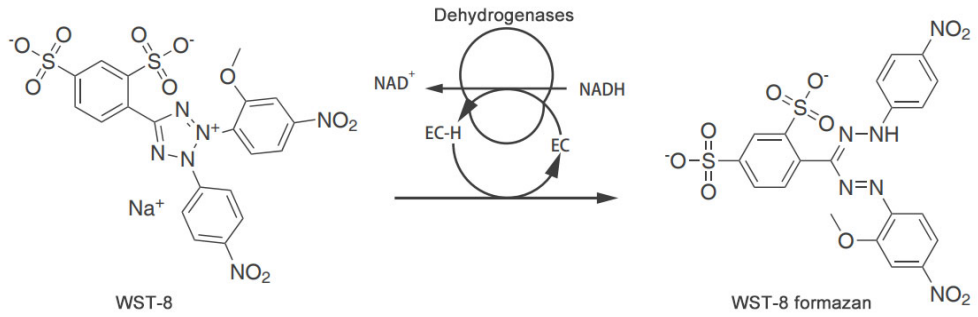
Quantitation of viable cell number in proliferation and cytotoxicity assays.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

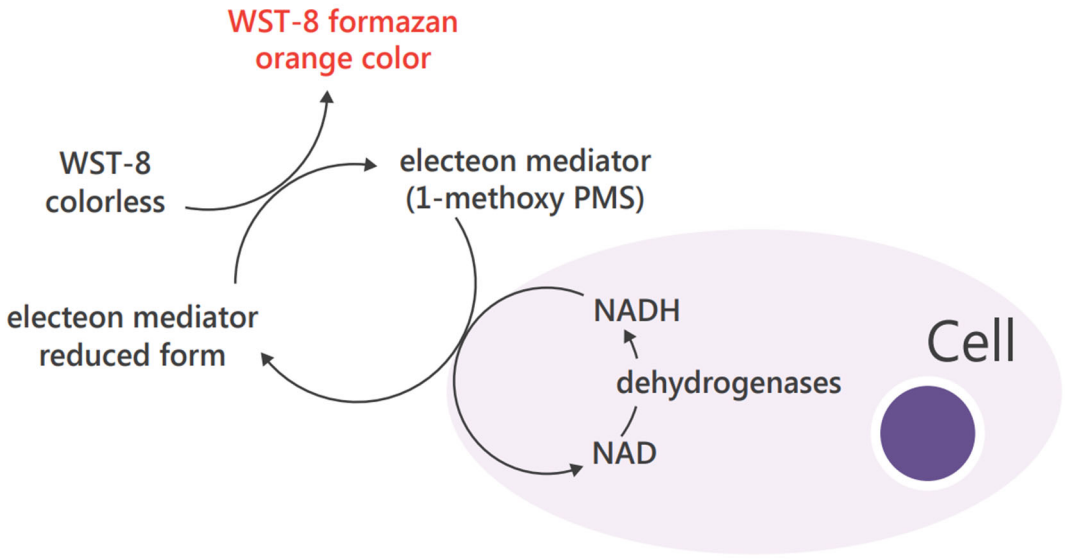
Cell Counting Kit-8 (CCK-8) provides a tool for studying induction and inhibition of cell proliferation in any in vitro model. Cell Counting Kit-8 (CCK-8) allows very convenient assays by utilizing highly water-soluble tetrazolium salt. WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4- disulfophenyl)-2H-tetrazolium, monosodium salt] produces a water-soluble formazan dye upon reduction in the presence of an electron mediator.

CCK-8 is a one-bottle solution, ready to use. CCK-8, being nonradioactive, allows sensitive colorimetric assays for the determination of the number of viable cells in cell proliferation and cytotoxicity assays.



II. PRINCIPLES

WST-8 is bioreduced by cellular dehydrogenases to an orange formazan product that is soluble in tissue culture medium. The amount of formazan produced is directly proportional to the number of living cells. Since the CCK-8 solution is very stable and it has little cytotoxicity, a longer incubation, such as 24 to 48 hours, is possible.



III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Plate reader (450 nm filter)
2. 96-well plate
3. CO_2 incubator
4. 10 µl, 100-200 µl and multi-channel pipettes

IV. ASSAY PROCEDURE

Cell Number Determination

1. Inoculate cell suspension (100 µL/well) in a 96-well plate. Pre-incubate the plate in a humidified incubator (e.g., at 37°C, 5% CO_2).
2. Add 10 µL 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.

Cell Proliferation and Cytotoxicity Assay

1. Seed cells in a 96-well plate at a density of 5000 cells/well in 100 µL of culture medium with or without compounds to be tested. Culture the cells in a CO_2 incubator at 37°C for 24 hours.
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-8 solution to each well of the plate using a repeating pipettor. 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. Before reading the plate, it is important to mix gently on an orbital shaker for 1 minute to ensure homogeneous distribution of color.
7. Measure the absorbance at 450 nm using a microplate reader.

V. CALCULATION

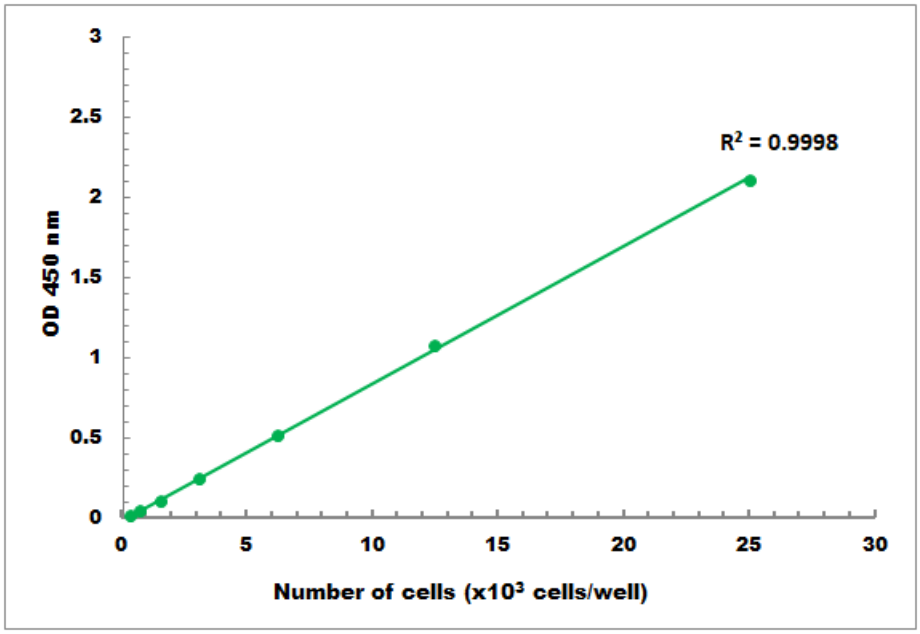
Cell survival rate = $[(OD_{sample} - OD_{blank}) / (OD_{control} - OD_{blank})] \times 100\%$

Cell inhibition rate = $[(OD_{control} - OD_{sample}) / (OD_{control} - OD_{blank})] \times 100\%$

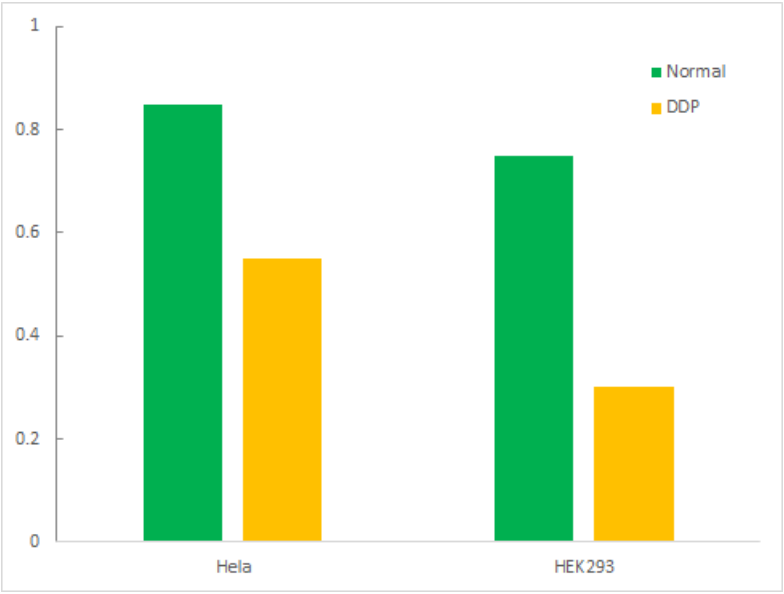
VI. STORAGE/STABILITY

The kit ships on wet ice and storage at 2-8 °C is recommended.

VII. EXAMPLE DATA



Cell line: Hela cells **Medium**: DMEM, 10% FBS **Incubation**: 37°C, 5% CO_2, 2 hours



Cell line: Hela, HEK293 **Medium**: DMEM, 10% FBS

Chemicals: 200 µM DDP **Incubation**: 37°C, 5% CO_2, 2 hours

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES