v.2024.02.16

Cat. No. 291

CellVia®

Cell Counting Kit 8 (CCK8) Assay Technical Manual

Quick Facts

1. Storage conditions before opening:<-20 ℃/ Protect from light.
2. Measure the absorbance at 450 nm.
3. Recommended operation conditions：
   Add 10 µl of the CCK-8 solution to each well of the plate containing 100 µl culture media and incubates 1~4 hrs. (37°C, 5% CO_2).
4. Do not use this product if the solution has turned into yellow color.

Information：

Cell Counting Kit-8 (CCK-8) allows very convenient assays by utilizing the 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, as shown in **Fig. 1**. CCK-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 and cytotoxicity assays. WST-8 is reduced by dehydrogenases in cells to give a orange-colored product (formazan), which is soluble in the 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 detection sensitivity of CCK-8 is higher than other tetrazolium salts such as MTT, XTT, MTS or WST-1.

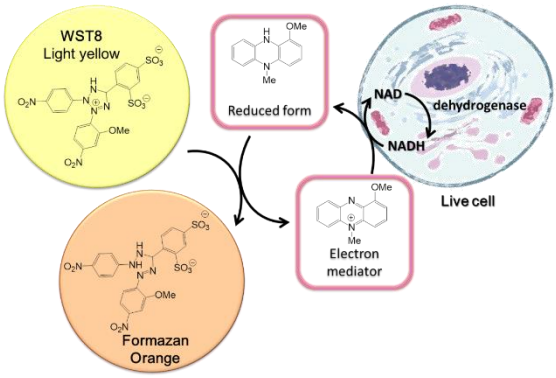

Fig. 1 Principle of the cell viability detection with CCK-8.

Protocol：

1. Inoculate cell suspension (100 µl/well) in a 96-well plate. Also prepare wells that contain known numbers of viable cells (to create a calibration curve in step 5). Pre-incubate the plate in a humidified incubator (e.g., at 37 °C, 5% CO2).
2. Thaw the CCK-8 on the bench top if it is frozen. *Note: It takes about 30 minutes on the bench top at 25 °C.*
3. Add 10 µl of the CCK-8 solution to each well of the plate. *Note: 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. Prepare a calibration curve using

the data obtained from the wells that contain known numbers of viable cells.

Background control：

Slight spontaneous absorbance around 450 nm occurs in culture medium incubated with CCK-8. This background absorbance depends on the culture medium, pH, incubation time and length of exposure to light. Typical background absorbance after 2 hours incubation is 0.1 - 0.2 absorbance units. To correct for this, prepare one or more control wells without cells, and subtract the average absorbance of the control wells from that of the other wells.

Advantages：

1. One-bottle, ready-to-use solution.
2. The toxicity of CCK-8 is so low that, after the CCK-8 assay is completed, the same cells can be used for other cell proliferation assays.
3. No harvesting, no washing and no solubilization steps
4. As showing in **Fig. 2**, CCK-8 has higher sensitivity than MTT or MTS.

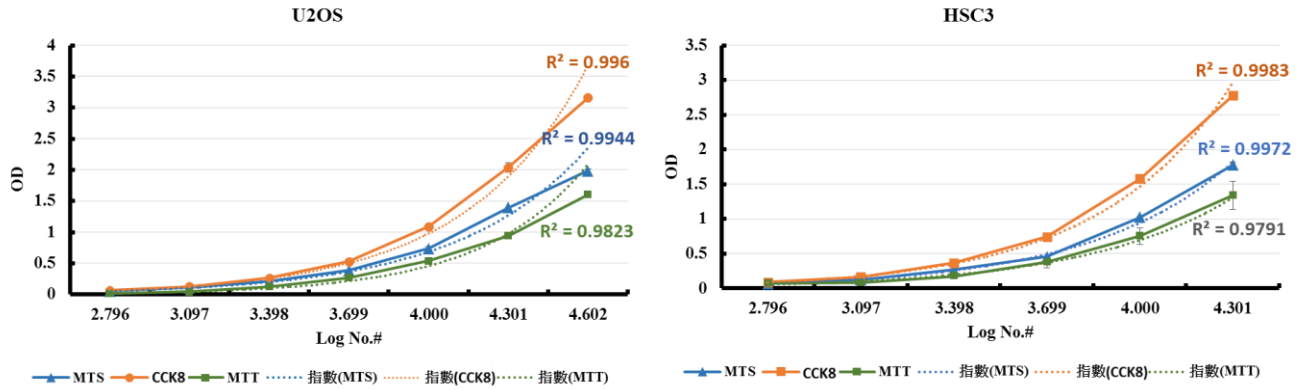

Fig. 2 Compare the sensitivity of MTT、MTS and CCK-8 assays

5. As shown in **Fig. 3**, the mouse nerve cells is used to compare our product with the commercially available one. The results show almost the same performance.

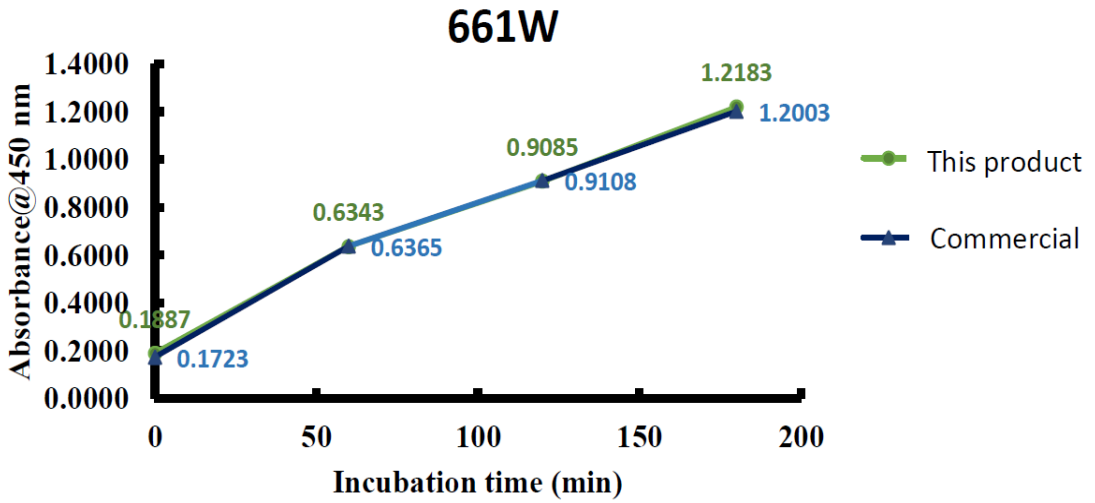

Fig. 3 The performance of our CCK-8 compares with commercial available product.

6. As shown in **Fig. 4**, the effect of the two drugs is tested with liver cancer cells. The cell proliferation of this product is similar to that of the commercially available products, and is superior to the traditional MTT assay.

Commercial

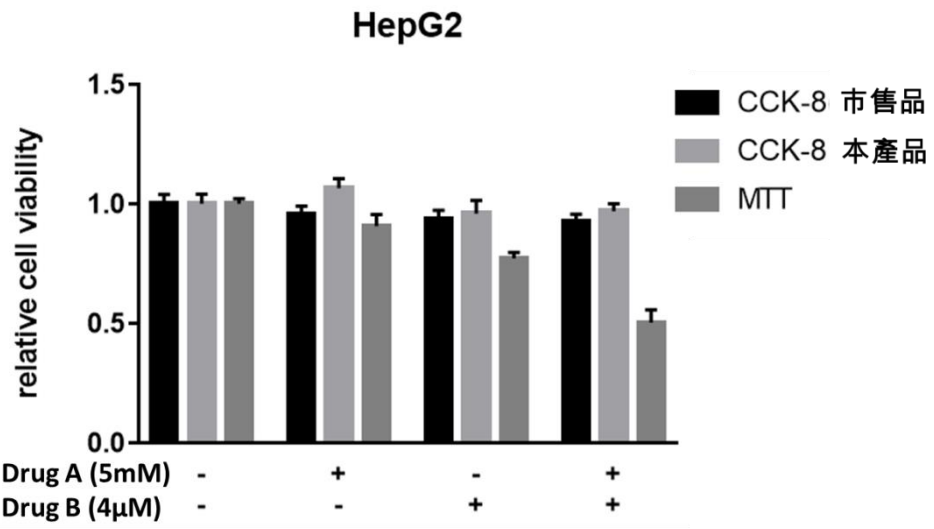

Fig. 4 The effect of two drugs on proliferation/toxicity of cancer cell.

Storage：

1. CCK-8 solution should keep its original red color and does not turn orange. If you utilize CCK-8 frequently, Keep it at 0-5 °C with protection from light, and finish within 1 week after opening.
2. CCK-8 is stable for 2 years at-20 ℃ with protection from light.

Precautions：

1. Repeated thawing and freezing causes an increase in the background, which interferes with the assay.
2. Keep CCK-8 protection from light all the time.

Reference：

1. Cell-Penetrating Delivery of Nitric Oxide by Biocompatible Dinitrosyl Iron Complex and Its Dermato-Physiological Implications. Int. J. Mol. Sci. 2021, 22(18), 10101.
2. Sneha Sundaran, Li-Ching Kok and Hwan-You Chang. Fabrication and in vitro evaluation of photo cross-linkable silk fibroin–epsilon-poly-L-lysine hydrogel for wound repair. Biomedical Materials. 2023.18. 5. 055021.

聚創新材料股份有限公司

IMT FORMOSA New Materials Co., Ltd.

電話 Phone: +886-926159317

電郵 Email: polycreatives@outlook.com

聯絡人 Contact: Peter Hsu