Cell Counting Kit-8 (CCK-8)

Cat #: orb1173259 (manual)

Product name: Cell Counting Kit-8 (CCK-8)

Catalog number: orb1173259

Storage: Storage at 4℃ for 12 months, protected from light. Storage at -20℃ for a more long time.

Assay Principle

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, 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 an orange-colored product (formazan), which is soluble in the tissue culture medium. Cell Counting Kit-8 (CCK-8) is designed to detect cell proliferation and cell toxicity based on WST-8 is reduced by dehydrogenases in cells to give an orange colored product (formazan). The amount of the formazan in cells is directly proportional to the number of living cells. The product (formazan) produced by WST-8 is water soluble, no organic solvents or isotopes required. And the formazan is stable and safe. The detection sensitivity using CCK-8 is higher than assays using other tetrazolium salts such as MTT, XTT, MTS or WST-1.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	1000 T	10000 T	
Ready-to-use CCK-8 solution	10 mL	100 mL	Storage at 4℃ for 12 months, protected from light. Storage at -20 ℃ for a more long time. Repeated freezing and thawing will increase the background and influence the detection. If you use it frequently, store the kit at 4℃.

Materials Required but Not Supplied

- Microplate reader capable of measuring absorbance at OD450 nm, humidifying carbon dioxide incubator (37℃，5% CO2)
- 96-well cell culture plate with clear flat bottom, precision pipettes, disposable pipette tips

Reagent Preparation

Ready-to-use CCK-8 solution: Ready-to-use, no premixing of components required.

Biorbyt Ltd
7 Signet Court, Swann Road, Cambridge, CB5 8LA. United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859-353 | Fax: +44 (0)1223 280-240

Biorbyt LLC
68 TW Alexander Drive, Durham, NC 27709. United States
Email: info@biorbyt.com | Phone: +1 (415) 906-5211 | Fax: +1 (415) 651-8558

Assay Procedure

Cell Number Determination Protocol

1. Inoculate cell suspension (100 µL/well) in a 96-well plate. Pre-incubate the plate in a humidified incubator (at 37°C, 5% CO2).
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 OD reading.
3. Incubate the plate for 1-4 h in the incubator. The incubation time depends on the experimental conditions such as cell type and cell density;
4. Measure the absorbance at 450 nm using a microplate reader.

Cell Proliferation and Cytotoxicity Assay Protocol

1. Dispense 100 µL of cell suspension (5,000 cells/well) in a 96-well plate. Pre-incubate the plate for 24 h in a humidifying carbon dioxide incubator (at 37°C, 5% CO2).
2. Add 1-10 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 h) 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, since they interfere with the OD reading.
5. Incubate the plate for 1-4 h in the incubator. The incubation time depends on the experimental conditions such as cell type and cell density;
6. Measure the absorbance at 450 nm using a microplate reader.

Data Analysis

Cell viability=$[(A_s-A_b)/(A_c-A_b)]\times100\%$

Inhibition rate=$[(A_c-A_s)/(A_c-A_b)]\times100\%$

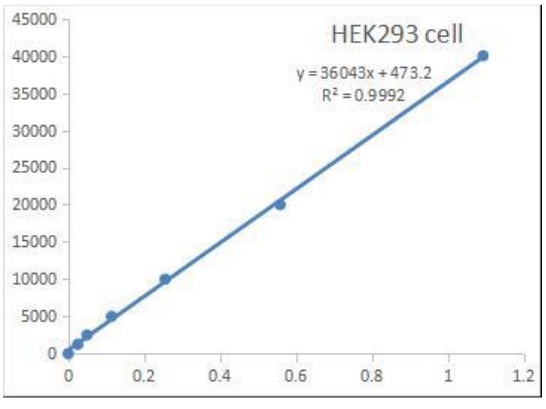
A_s: Absorbance of experimental wells (including cells, culture medium, CCK-8 solution and drug solution);

A_c: Absorbance of control wells (including cells, culture medium, CCK-8 solution, without drug);

A_b: Absorbance of blank wells (including culture medium, CCK-8 solution, without cells and drugs).

Typical Data

Typical standard curve:



OD450-Blank

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Figure 1. The CCK-8 kit for 96-well plate analysis detects cell viability. Data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Precautions

1. 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.
2. Be careful not to introduce bubbles to the wells, since they interfere with the OD reading.
3. 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 h) or a large number of cells (~10^5 cells/well) may be necessary.
4. If the color or pH of culture media is changed due to long-time culture, please change the culture media when adding CCK-8.
5. The same cells can be used for other cell assays because of the low toxicity of CCK-8.
6. The OD value range usually is 0.1-2.0, and it is better around 1.0. Generally, the OD value of the control wells (including cells, culture medium, CCK-8 solution, without drug) can be controlled at about 0.8-1.0 to determine the number of cells tested and the incubation time.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

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