Cell Counting Kit-8 (CCK-8)

Description

The Bimake Cell Counting Kit-8 (CCK-8) is a ready-to-use one-bottle solution which offers a simple, rapid, reliable and sensitive measurement of cell viability and the cytotoxicity of agents within various chemicals quantitatively. Moreover, the Bimake Cell Counting Kit-8 (CCK-8) is very safe, fast, nonradioactive and cost-effective compared to other conventional methods such as [3H]-thymidine incorporation assay.

The Bimake Cell Counting Kit-8 (CCK-8) is a redox indicator that utilizes the highly water-soluble tetrazolium salt WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] to produce a water-soluble formazan dye upon reduction by cellular dehydrogenases in the presence of an electron carrier 1-Methoxy PMS in the culture medium, as shown in Figure 1. 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 Cell Counting Kit-8 (CCK-8) is higher than other tetrazolium salts such as MTT, XTT, MTS or WST-1.

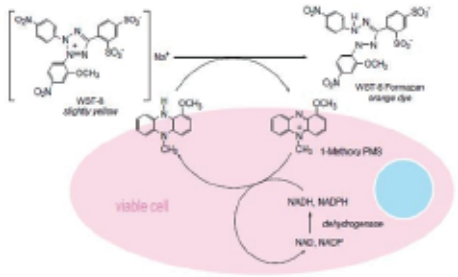

Figure 1: Working mechanisms of Cell Counting Kit-8 (CCK-8)

Customer Reviews

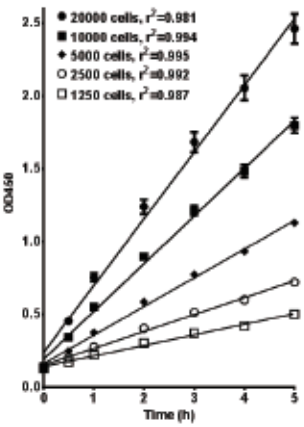

Figure 2: Cell number vs OD value using Cell Counting Kit-8 (CCK-8). K562 cells were seeded into 96-well plate at different density in 100 µl culture medium in triplicate respectively. 16 hours later, 10 µl/well Cell Counting Kit-8 (CCK-8) were added, allowing to continuously culture for indicated duration in cell culture incubator. The absorbance at 450 nm was then measured in a microplate reader. Medium only wells were served as blank control. Note the great linear relationship between cell number per well versus OD450 values at all incubation time.

Figure 3: Time dependent color development using Cell Counting Kit-8 (CCK-8). HEK293 cells were seeded into 96-well plate at different concentrations in 100 µl culture medium in triplicate. 16 hours later, 10 µl/well Cell Counting Kit-8 (CCK-8) was added, allowing cells to continuously culture for indicated duration in cell culture incubator. Absorbance at 450 nm was then measured in a microplate reader. Note the highly linear relationship of color development versus incubation time at all cell concentrations.

Components

Contents	Cat#:B34302	Cat#:B34304
Unit size	5 ml	25 ml

Experimental Protocol

1. Required Materials (not included)

- microplate reader (450 nm filter).
- 10 µl, 100-200 µl and multi-channel pipettes.
- 1 % SDS (optional).

2. Experiment Procedure

1) Inoculate cells in a cell culture flask or dish, and allow cells to adhere or grow for approximately 4–24 hours before proceeding with the assay.

2) Add 1/10 volume of Cell Counting Kit-8 (CCK-8) directly to cells in culture medium. Mix thoroughly to achieve a homogenous solution by lightly tapping the outside of the plate several times while avoiding bubbles. For 96-well plate, add 10 µl Cell Counting Kit-8 (CCK-8) per 100 µl culture medium.

3) Incubate in a cell culture incubator for 0.5 to 4 hours at 37°C until the color turns orange. Over incubation will give false results.

Note: Sensitivity of detection increases with longer incubation times. For samples with fewer cells, use a longer incubation time of up to 24 hours.

4) Record results using microplate reader to measure the absorbance of Cell Counting Kit-8 (CCK-8) at 450 nm. Recommended OD values range between 0.8-1.5, however values between 0.5-2.5 are acceptable.

Note: Slight spontaneous absorbance around 450 nm occurs in culture medium incubated with Cell Counting Kit-8 (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.

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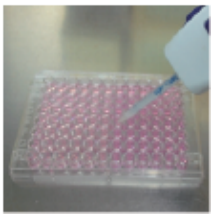
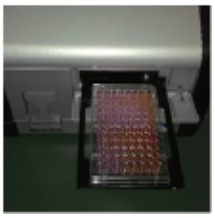
5) Optional: Add 10 μl of 1 % SDS (dissolve 0.1 g SDS with PBS buffer to prepare 10 ml solution) directly to 100 μl of cells to stop the reaction. Signals can be read within 3 days without affecting the absorbance values.
6) Calculation of cell viability:
Cell viability (%) = [A (Drug+) – A (Black)] / [A (Drug-) – A (Black)] x 100%
A (Drug+) : OD value of wells with cells, CCK-8 and drugs;
A (Drug-) : OD value of wells with cells, CCK-8, but without drugs;
A (Black): OD value of wells with culture medium and CCK-8, but without cells.

3. Brief Summary of the protocol



Add 10% (v/v) Cell Counting Kit-8 (CCK-8)

Culture for 0.5-4 hours



Record OD450nm

Storage

The Bimake Cell Counting Kit-8 (CCK-8) is stable for 6 months at room temperature, 2 years at 0-5 °C with protection from light. For long term storage, keep it at -20 °C and below. Repeated thawing and freezing cycles are not recommended because of an increase in the background, which interferes with the assays. Please store the reagent at 4 °C for daily use.

Note

1. Cell Counting Kit-8 (CCK-8) is ready-to-use solution. Mix the reagent to ensure a homogenous solution before use.
2. If you plan to use longer incubation time (overnight), be sure to maintain sterile conditions during reagent addition and incubation to avoid microbial contaminants. Contaminated cultures will yield erroneous results as microbial contaminants also reduce the Cell Counting Kit-8 (CCK-8).
3. Be sure to include appropriate assay controls. To minimize experimental errors, we recommend making measurements from a minimum of 3–8 replicates of experimental and medium only control.
4. This product is for R&D use only, not for medical, household, or other uses.

Trouble Shooting

Problem	Potential Cause (s)	Suggestion
OD values are too high (>2.5)	Too many cells in a well.	Reduce the cell count. Pre-determination of the relationship between cell number and fluorescence readings is recommended.
	Incubation time is too long.	Shorten the incubation time.
OD values are too low (<0.3)	Too little cells per well.	Increase the cell count. Pre-determination of the relationship between cell number and O.D readings is recommended.
	Cell viability is too low to be tested by Bimake Cell Counting Kit-8 (CCK-8).	Increase cell number per well and incubation time. If it not work again, choose other more sensitive kits.
	Incubation time is too short.	Increase the incubation time, up to 24 h is acceptable.
	Wrong wave length of reading is used.	Use the optical filter or dual wavelength determination (the detection wavelength is 430-490nm and the reference wavelength is 600-650nm).
Color is developed by dead cells.	Test compounds may reduce WST-8	If color development occurs by mixing and incubating of Bimake Cell Counting Kit-8 (CCK-8) and test compounds, exchange the culture media and then add Bimake Cell Counting Kit-8 (CCK-8)
	Cell lysate may reduce WST-8.	Exchange fresh culture media and then add Bimake Cell Counting Kit-8 (CCK-8).
Date vary between wells	It's not thoroughly mixed after adding Cell Counting Kit-8 (CCK-8) to each well.	Exchange fresh culture media and then add Bimake Cell Counting Kit-8 (CCK-8), mix thoroughly by lightly tap the outside of the well and incubate again.
	Bimake Cell Counting Kit-8 (CCK-8) is not well mixed after long term storage such as freezing and thawing.	Mix Bimake Cell Counting Kit-8 (CCK-8) thoroughly, exchange fresh culture media and the re-add Bimake Cell Counting Kit-8 (CCK-8).
	Because of the bubbles in wells.	Pierce bubbles by a toothpick.
	Cells are various among wells.	Re-seed cells and repeat the whole assay.
	The edge effect of a plate.	Do not use the outer-most wells for the assay. Just add media to these wells.
The absorbance of the well with a test compound is higher than that of the negative control.	The compound promotes cell viability even though it is toxic substance.	Bimake Cell Counting Kit-8 (CCK-8) still works bona fide in such situation. Change other assay kit with different principles.
High background (blank) absorbent values	The reagent may be breaking down due to multiple freeze/thaw cycles.	Be sure to store Bimake Cell Counting Kit-8 (CCK-8) at 0-5°C in the dark place. Don't use the expired products.

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