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Catalog No.C0005

Cell Counting Kit-8 (CCK-8)

Instruction Manual

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Product Description

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] which produces a water - soluble formazan dye upon reduction in the presence of an electron carrier. Cell Counting Kit-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.

Advantages

- More sensitive than MTT, MTS or WST-1
- No toxicity to cells
- Simple steps; No organic solvents required
- Stable and ready to use

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Assay Mechanism

WST-8 is reduced by dehydrogenases in cells to give a yellowcolored product (formazan), which is soluble in the tissue 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.

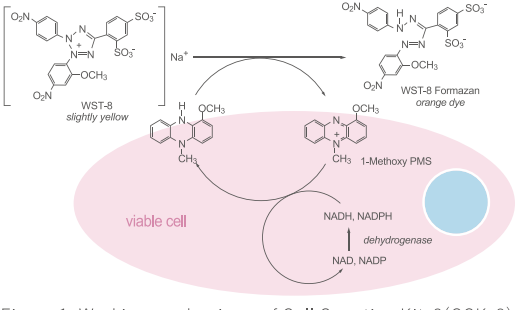

Figure 1: Working mechanisms of Cell Counting Kit-8(CCK-8).

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Official Website
https://www.targetmol.com

(781) 999-4286

sales@targetmol.com

34 Washington Street, Suite 220,
Wellesley Hills, Massachusetts 02481

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Properties	MTT	XTT	WST-1	CCK8
Solublity of formazan	-	+	+	+
Forms	Power	2-bottle solution	1-bottle solution	1-bottle solution
Preparation	Dissolve before use	Mix before use	Ready to use	Ready to use
Sensitivity	+	++	++	+++
Detection Speed	+	++	++	+++
Wavelength	560~600nM	420~480nM	420~480nM	430-490nM
Toxicity	+	-	-	-
Stability	+	-	+	++
96-Well Plate Compatibility	+	++	++	++
Convenience	+	++	++	+++

Applications

- Cell proliferation determinations
- Cytokine assays
- Cell viability assays
- Cytotoxicity assays

Note

1. Cell Counting Kit-8 (CCK-8) is ready-to-use solution. Mix the reagent to ensure a homogenous solution before use.
2. Pay attention to the edge effect of 96-well plate. It is suggested to discard the surrounding plate hole and add the same amount of PBS.
3. This product is for R&D use only, not for medical, household, or other uses.

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Experiment Procedure

1. 100uL cell suspension was inoculated on 96-well plate and incubated in cell incubator (37 °C, 5% CO2).
2. Take the cells out of the incubator, 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 ul Cell Counting Kit-8 (CCK-8) per 100 ul culture medium.
3. Incubate in a cell culture incubator for 1 to 4 hours at 37 °C until the color tums orange. Over incubation will give false results.
4. Place the 96-well plate on the shaking table for about 1min before reading with the micropalte reader to ensure the uniform color of orifice plate.
5. The 450 nm light absorption value was read by an enzyme marker and cell activity was calculated.
6. Optional: Add 10 ul of 1% SDS (dissolve 0.1 g SDS with PBS buffer to prepare 10 ml solution) directly to 100 ul of cells to stop the reaction. Signals can be read within 3 days without affecting the absorbance values.

Storage

The Cell Counting Kit-8 (CCK-8) is stable for 1 years at 4 °C with protection from light. For long term storage, store at -20 °C and below.