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**(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)**

## Cell Counting Kit 8 (WST-8 / CCK8)

**Catalog No:** E-CK-A361

**Size:** 100 Tests / 500 Tests / 1000 Tests

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<b>Cat.</b>	<b>Products</b>	<b>100 Tests</b>	<b>500 Tests</b>	<b>1000 Tests</b>	<b>Storage</b>
E-CK-A361	CCK-8 Buffer	1 mL	5 mL	10 mL	-20°C
<b>Manual</b>			<b>One Copy</b>		

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This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)

Email: [techsupport@elabscience.com](mailto:techsupport@elabscience.com)

Website: [www.elabscience.com](http://www.elabscience.com)

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

## Introduction

Elabscience® Cell Counting Kit 8 (WST-8 / CCK8) is a rapid and highly sensitive kit based on WST-8, which is widely used in cell proliferation and cytotoxicity detection.

## Detection principle

WST-8 is a compound similar to MTT, which can be reduced to orange formazan by some dehydrogenase in mitochondria in the presence of electron coupling reagent. The more and faster the cells proliferate, the darker the color is, and the more cytotoxic, the lighter the color is. For the same cells, there is a linear relationship between the depth of color and the number of cells.

## Experimental Procedure

1. Add 100  $\mu$ L (about 2,000 cells) of cell suspension per well (Two blank group wells were added with the same volume of culture medium without cells), incubate at 37  $^{\circ}$ C, 5% CO<sub>2</sub> Cell incubator for 24 h.

**Tips:** A. For cell proliferation test, add 100  $\mu$ L (about 2,000 cells) to each well, for cell cytotoxicity test, add 100  $\mu$ L (about 5,000 cells) to each well (The number of cells used in each pore depends on the size of the cell and the rate of cell proliferation, etc.)

B. The incubate temperature is different depending on cell types, most mammalian cells are recommended at 37  $^{\circ}$ C. Other species please choose the appropriate temperature according to cell culture conditions.

2. Add 10  $\mu$ L specific drug stimulation to each well to stimulate the cells (Two negative control wells contain cells were added with the same volume of culture medium without drugs).
3. Incubate at 37  $^{\circ}$ C, 5% CO<sub>2</sub> and 100% humidity Cell incubator for appropriate time.  
**Tip:** Ditto. Selection of appropriate incubation conditions and time according to different cell type
4. Add 10  $\mu$ L CCK-8 Buffer, incubate at 37  $^{\circ}$ C, 5% CO<sub>2</sub> Cell incubator for 1~4 h.  
**Tip:** Ditto. Selection of appropriate incubation conditions and time according to different cell type
5. Measure the absorbance at 450 nm, if there is no 450 nm filter, the 420 nm~480 nm filter can be used.

6. Result Analysis:

A. Cell Survival Rate. The OD value of each test well was subtracted from the background OD value (blank group), and the OD value of each repetitive well was taken as an average ( $\pm$ SD).

Cell Survival Rate % = (drug stimulation cells OD / negative control cells OD)  $\times$  100%.

B. Calculated when T/C = 50%, the drug stimulation concentration (IC<sub>50</sub>) and T/C = 10% the drug stimulation concentration (IC<sub>90</sub>).

**Tip:** It is suggested that the wells with culture medium and CCK-8 solution but without cell is used as blank control.

If you are worried that the drugs will interfere with the result, please set the wells with cell culture medium, drugs and CCK-8 solution but without cells as blank control.

## Storage

Store at 2~8 $^{\circ}$ C for one year in dark. For long time storage, please store at -20 $^{\circ}$ C and the shelf life can

be two year

## Cautions

1. For long time storage, please store at  $-20^{\circ}\text{C}$ . For ordinary usage, please store at  $2\sim 8^{\circ}\text{C}$ . Avoid freeze / thaw cycles.
2. Mixing the cell suspension before adding to the wells to avoid cell precipitation leading to different cell number in each well.
3. The optimum reaction time of CCK-8 depends on the specific optimum time of colour development. It is suggested that the optimum cell number and incubation time of CCK-8 should be explored by pre-experiment CCK-8.
4. The detection of this kit depends on dehydrogenase-catalyzed reactions, so reductants (such as some antioxidants) may interfere with the detection. If there are more reductants in the system to be detected, try to remove them.
5. For your safety and health, please wear the lab coat and disposable gloves before the experiments.