

# Cell Counting Kit-8 (CCK8) Cell Viability Assay Kit

Catalog No.: abx090677

Revision date: 20-Jul-17

Size: 500 tests

Storage: Store at 4 °C in the dark for up to one year, or store at -20 °C in the dark for up to two years.

#### Introduction

Abbexa's Cell Counting Kit-8 (CCK8) is a WST-8 based cell viability assay kit, which is widely used for cell proliferation and cytotoxicity assays. This kit can be used for cytokine-induced cell proliferation assays, anti-carcinogen induced cell cytotoxicity assays or drug induced cell growth inhibition assays. This kit is a one-bottle solution, which does not require mixing of any components. All assays can be carried out in one 96-well plate without washing and can therefore be used to analyze samples in bulk.

#### Principle of the assay

WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium), combined with PMS electron mediator, is reduced by dehydrogenases found in cell mitochondria to yield a water-soluble formazan dye. The faster the cell proliferation, the deeper of the color. The greater the cell cytotoxicity, the lighter the color. In addition, since WST-8 is not toxic to cells, the incubation times with WST-8 are flexible and adaptable to many different cell types. Phenol red and serum do not influence the performance of this kit.

### Procedure

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- 1. Seed 100 μl of cells in a 96-well plate at a density of 1000-10,000 cells per well. Add 100 μl of PBS to 2 wells as a negative control.
- 2. Culture the cells in a 5% CO<sub>2</sub> incubator at 37°C until the cells reach confluence. Add 0-10 μl of the substance of interest into each well. Incubation times should be determined by the end user.
- 3. Add 10 µl of CCK8 into each well, including the control wells. Gently tap the plate to ensure thorough mixing.

Incubate for 0.5-4 hours.

**Note:** For most samples, incubation for 1 hour is sufficient. The incubation time should be optimized depending on the cell type and concentration. We recommend that O.D. values are taken after incubating for 30 minutes, 1 hour, 2 hours and 4 hours to choose the optimal time for future assays.

5. Gently tap the plate to ensure thorough mixing. Measure the absorbance at 450 nm.

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# **Product Manual**

## Notes

- 1. Long assay times may lead to evaporation of liquid from the wells. Avoid using the outermost wells and use PBS buffer, water or culture medium to replace any evaporated liquid.
- 2. This kit is based on the reduction of WST-8 by dehydrogenase enzymes. Other reducing agents such as antioxidants may interfere with the kit.
- 3. Ensure that there are no bubbles present in the wells before measuring the absorbance.
- 4. Personal protective equipment (PPE), such as lab coats, disposable gloves and eye protection, should be used when carrying out the assay.