Instructions for Use
Revision date: 6-Oct-20

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

Catalog No.: abx090677

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

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_2 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.
4. 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.

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.