Cell Counting Kit 8 (CCK-8)

Catalog number: 22772, 22773
Unit size: 1000 Tests, 10000 Tests

Component	Storage	Amount (Cat No. 22772)	Amount (Cat No. 22773)
CCK-8 Solution	Freeze (< -15 °C), Minimize light exposure	1 bottle (10 mL)	1 bottle (100 mL)

OVERVIEW

Cell Counting Kit 8 (CCK-8) is a colorimetric assay used to quantitate cell proliferation, cell viability and cytotoxicity.

CCK-8 utilizes the enzymatic activity of live cells to reduce a colorless compound into an orange dye, formazan. The amount of formazan produced, as determined by absorbance ($\lambda_{460\ nm}$), is directly proportional to the number of live cells in the sample.

Metabolically active cells are known to produce dehydrogenase enzymes. Under neutral pH conditions and with the aid of an electron acceptor known as 1-methoxy phenazine methosulfate, NADPH-dependent cellular enzymes operate across the cell membrane to mediate the reduction of the colorless, cell-impermeant WST-8 tetrazolium salt. This yields an orange-colored formazan dye which is soluble in cell culture media or aqueous buffers, exhibiting strong absorption at 460 nm. This absorbance is directly proportional to the number of live cells present in the samples, serving as a reliable indicator of cell viability.

AT A GLANCE

Protocol Summary

1. Prepare cells in a 96-well plate (100 µL/well).
2. Add 10 µL of CCK-8 Solution to each well.
3. Incubate at 37°C for 1 - 4 hours.
4. Monitor absorbance at OD = 460 nm.

Important Note

The CCK-8 Solution can be stable for up to one year if stored at 4°C and protected from light. Store it at <-20°C for longer storage.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	460 nm
Recommended plate	Clear bottom

CELL PREPARATION

For guidelines on cell sample preparation, please visit:

https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

Cell Proliferation and Cytotoxicity Assay:

1. Plate 5000 to 10,000 cells/well in a tissue culture microplate with clear bottom.
2. Add test compounds into the cells and incubate for a desired period of time (such as 24, 48, or 96 hours) in a 37°C, 5% CO_2 incubator. For blank wells (medium without the cells), add the same amount of test compounds. The suggested total volume is 100 µL for a 96-well plate or 50 µL for a 384-well plate.

   Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for proliferation or cytotoxicity induction. For proliferation assays, use fewer cells; for cytotoxicity assays, use more cells to start with.
3. Add 10 µL/well (96-well plate) or 5 µL/well (384-well plate) of CCK-8 Solution to each well.
4. Incubate the plate at 37°C for 1 - 4 hours, protect from light.

   Note: The incubation time could be from 30 minutes to overnight depending on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.
5. Monitor the absorbance increase with an absorbance plate reader at OD =460 nm.

Cell Counting Assay:

1. Prepare cell culture in a tissue culture microplate with a clear bottom. The suggested total volume is 100 µL for a 96-well plate or 50 µL for a 384-well plate.

   Note: We used serially diluted HeLa and Jurkat cell suspension in a clear bottom 96-well plate for the assay.
2. Add 10 µL/well (96-well plate) or 5 µL/well (384-well plate) of CCK-8 Solution to each well.
3. Incubate the plate at 37°C for 1 - 4 hours, protect from light.

   Note: The incubation time could be from 30 minutes to overnight depending on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.
4. Monitor the absorbance increase with an absorbance plate reader at OD = 460 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

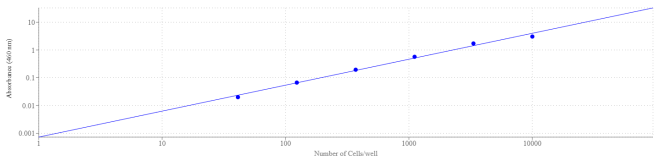

Figure 1. Cell number was determined with Cell Counting Kit 8 (CCK-8). HeLa cells at 40 to 10,000 cells/well/100 µL were added in a clear bottom 96-well plate. The absorbance was measured at 460

nm using a SpectraMax reader (Molecular Devices).

DISCLAIMER

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