TOOLS Cell Counting Kit-8 (CCK-8)

Catalog No. TEN-CCK8 / TEN-CCK8-100

Product Size: 5 mL (500 assays) / 100 mL (10000 assays)

Storage: 4 °C for 1 year

Introduction

TOOLS Cell Counting Kit-8 (CCK-8) is a ready-to-use solution that does not require the premixing of components. CCK-8, which is nonradioactive, allows sensitive colorimetric assays to be performed to determine the number of viable cells in cell proliferation and cytotoxicity assays. WST-8 is reduced by dehydrogenases in cells to form a yellow product (formazan) that is soluble in the tissue culture medium. The amount of 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 that of other tetrazolium salts, such as MTT, XTT, MTS, and WST-1.

The kit components are sufficient for performing up to 500 or 10000 assays.

Protocol

A. Cell Proliferation Assay

1. Inoculate the cell suspension (100 µL/well) in a 96-well plate, and prepare wells that contain known numbers of viable cells (to create a calibration curve in step 4). Preincubate the plate in a humidified incubator (e.g., at 37 °C with 5% CO_2).
2. Add 10 µL of the CCK-8 solution to each well of the plate.

Note: Be careful not to introduce bubbles into the wells because they interfere with the OD reading.

3. Incubate the plate for 1–4 hours in the incubator.
4. Measure the absorbance at 450 nm by using a microplate reader. Prepare a calibration curve by using the data obtained from the wells that contain known numbers of viable cells.

Note: To later measure the absorbance, add 10 µL of 1% w/v sodium dodecyl sulfate (SDS) to each well, cover the plate, and store it with protection from light at room temperature. No absorbance change should be observed for 48 hours.

B. Cytotoxicity Assay

1. Add 100 µL of cell suspension (5000 cells/well) to a 96-well plate.
2. Preincubate the plate for 24 hours in a humidified incubator (e.g., at 37 °C with 5% CO_2).
3. Add 10 µL of various concentrations of toxicants into the culture media in the plate.
4. Incubate the plate for an appropriate length of time (e.g., 6, 12, 24, or 48 hours) in the incubator.
5. Add 10 µL of CCK-8 solution to each well of the plate.

Note: Be careful not to introduce bubbles into the wells because they interfere with the OD reading.

6. Incubate the plate for 1–4 hours in the incubator. Measure the absorbance at 450 nm by using a microplate reader.

Note: To later measure the absorbance, add 10 µL of 1% w/v SDS to each well, cover the plate, and store it with protection from light at room temperature. No absorbance change should be observed for 48 hours.

FAQs

1. How many cells should there be in a well?

For adhesive cells, at least 1000 cells are required per well (100-µL medium) when using the kit's standard 96-well plate. For leukocytes, at least 2500 cells are required per well (100-µL medium) because of low sensitivity. The recommended maximum number of cells per well for the 96-well plate is 25 000. If a 24- or 6-well plate is used for the CCK-8 assay, please calculate the number of cells per well and adjust the volume of the CCK-8 solution in a well to 10% of the total volume.

2. Does CCK-8 stain viable cells?

No, it does not stain viable cells because a water-soluble tetrazolium salt (WST-8) is used in the CCK-8 solution. The electron mediator, 1-methoxy PMS, receives electrons from a viable cell and transfers the electron to WST-8 in the culture medium. Because the formazan dye is also highly water-soluble, CCK-8 cannot be used for cell staining.

3. Does phenol red affect the assay?

No. The absorption value of phenol red in a culture medium can be removed by subtracting the absorption value of a blank solution from the absorption value of each well. Therefore, a phenol-red-containing medium can be used for the CCK-8 assay.

4. Is CCK-8 toxic to cells?

The toxicity of CCK-8 is so low that after the CCK-8 assay is completed, the same cells can be used for other cell proliferation assays, such as the crystal violet assay, neutral red assay, and DNA fluorometric assay.

5. I do not have a 450-nm filter. What other filters can I use?

You can use filters with absorbance values between 450 and 490 nm; however, the 450-nm filter provides the highest sensitivity.

6. Can I use CCK-8 for 384-well plates?

CCK-8 can be used for 384-well plates. Please dilute the CCK-8 solution by using phosphate buffered saline. The required volume of the CCK-8 solution is 5 µL per well for the 384-well plate.