Lumiprobe
Life science solutions

Assaying Cell Proliferation and Viability with Cell Counting Kit 8 (CCK-8)

Cell Counting Kit 8 (CCK-8) is a sensitive colorimetric assay used in biomedical research to determine cell viability, proliferation, and cytotoxicity. It works by employing the highly water-soluble tetrazolium salt, WST-8, which is reduced by intracellular dehydrogenases in live cells into a water-soluble, orange-colored formazan dye. The intensity of this color, measured by a microplate reader at a specific wavelength (around 450 nm), is directly proportional to the number of metabolically active cells.

The CCK-8 assay offers ease of use, minimal toxicity, and enhanced sensitivity compared to tests based on other tetrazolium salts such as MTT, XTT, MTS, or WST-1.

Cell Number Determination

To enable the quantification of absolute cell numbers in subsequent assays, a standard curve correlating the optical density (OD) with the cell count must be established under consistent experimental conditions.

1. Count the cell number in the suspension using a cytometer. Seed cells into a 96-well plate at the calculated density.
2. Serially dilute the cell suspension in culture medium to generate a gradient of cell concentrations. A minimum of 3 to 5 distinct concentrations is recommended; each replicated in 3 to 6 wells.
3. Culture cells until they have adhered to the wall. Subsequently, add CCK-8 reagent to each well and incubate for the predetermined duration.
4. Measure the absorbance at 450 nm.
5. Construct a standard curve by plotting the mean OD value (Y-axis) against the corresponding known number of seeded cells (X-axis). This curve allows the determination of the cell number in an unknown sample cultured at identical assay conditions (e. g., incubation time post-CCK-8 addition, etc.).

Cell Viability Assay

1. Seed cell suspensions (100 µL/well) in a 96-well plate.
2. Pre-incubate the plate for 24 h in an incubator at 37 °C and 5% CO_2.
3. Add 10 µL of CCK-8 reagent to each well, avoiding the introduction of air bubbles, as they can interfere with absorbance readings.
4. Incubate the plate for an additional 2 h.
5. Measure the absorbance at 450 nm using a microplate reader.
6. *(Optional)* If the absorbance cannot be measured immediately, the reaction may be stabilized by adding 10 µL of 0.1 M HCl or 1% (w/v) SDS solution to each well. The plate should then be covered and stored in the dark at room temperature; the absorbance values remain stable for up to 24 h under these conditions.

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Cell Proliferation and Cytotoxicity Assay

1. Seed cell suspensions (100 µL/well) in a 96-well plate.
2. Pre-incubate the plate for 24 h in an incubator at 37 °C and 5% CO_2.
3. Add 10 µL of test compound at various concentrations to the respective wells.
4. Incubate the plate for the desired experimental duration.
5. Add 10 µL of CCK-8 reagent to each well, avoiding bubble formation.
6. *(Optional)* If the test compound possesses inherent oxidative or reductive properties, the culture medium should be replaced with fresh, drug-free medium before adding CCK-8 to prevent artifactual results. For this, aspirate the existing medium, wash the wells twice with PBS or fresh medium, and then add 100 µL of new medium before the CCK-8 addition.
7. Incubate the plate for 2 h and measure the absorbance at 450 nm.
8. *(Optional)* If the absorbance cannot be measured immediately, the reaction may be stabilized by adding 10 µL of 0.1 M HCl or 1% (w/v) SDS solution to each well. The plate should then be covered and stored in the dark at room temperature; the absorbance values remain stable for up to 24 h under these conditions.

Cell Viability Calculation

Cell viability is expressed as a percentage and calculated using the following formula:

$$Cell\ Viability\ (\%) = [A\ (treated) - A\ (blank)] / [A\ (untreated) - A\ (blank)] \times 100$$

Where:

- *A (treated)* is the absorbance in the well containing cells, CCK-8, and the test compound.
- *A (blank)* is the absorbance in the well containing medium and CCK-8 only (no cells).
- *A (untreated)* is the absorbance in the well containing cells and CCK-8 only (no test compound).

This calculation yields a value representing either cell proliferative activity or cytotoxic viability.

Notes and Technical Considerations

- The CCK-8 assay is based on a dehydrogenase-catalyzed reduction reaction. Consequently, reducing agents and antioxidants present in the sample may confound the results and must be removed before analysis.
- Preliminary experiments are strongly recommended to optimize key parameters, such as the density of seeded cells and the incubation period following CCK-8 addition.
- Leukocytes and other non-adherent cell types may require extended culture times for adequate signal development.
- For adherent cells in a standard 96-well plate, a minimum seeding density of 1,000 cells per well (in 100 µL medium)

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is advised. Due to lower assay sensitivity for leukocytes, a minimum of 2,500 cells per well is recommended.

- When using different plate formats (e. g., 24-well or 6-well), scale the cell number and the volume of CCK-8 reagent proportionally, maintaining the CCK-8 volume at 10% of the total medium volume in the well.
- Although the optimal absorbance maximum is 450 nm, filters within the range of 430–490 nm are acceptable, albeit with reduced sensitivity.
- The background absorbance contributed by Phenol Red in the culture medium is automatically accounted for and subtracted during the calculation step via the blank well controls.
- The presence of air bubbles in wells significantly alters absorbance measurements and must be removed before reading the plate.
- Standard personal protective equipment (PPE), including safety glasses, gloves, and a laboratory coat, must be worn throughout this procedure.

Storage Conditions

- Store at 0–5 °C. Transport: Up to 21 days at temperatures up to 25 °C.
- CCK-8 is stable for one year when stored at 0–5 °C, protected from light.
- For more extended storage, freeze and store at -20 °C.
- Avoid repeated thawing and freezing, as this increases background levels, which interfere with assay results.
- Expiration Date: 12 months from date of shipment.

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