

CCK8

Generating a standard curve

1. Count the number of cells in your prepared cell suspension using a cytometer.
2. Dilute your cell sample with media to form a sequential cell concentration gradient in sequence, generally 3-5 cell concentration gradients, 3-6 multiple wells per group.
3. Incubate your samples for 2-4 hours.
4. Add CCK-8 and incubate for 1-4 h. Measure the OD value of your samples.
5. Plot the OD results on the Y-axis and the corresponding cell counts on the X-axis to generate a standard curve. This line can determine the number of cells in unknown samples.

Cell viability test

1. Add cell suspension (100 μ L/well) to a 96-well plate. Pre-incubate the plate in a humidified incubator (37°C, 5% CO₂).
2. Add 10 μ L of CCK-8 solution to each well.
Note: Be careful not to generate bubbles in the well, they will affect the reading of the OD value
3. Incubate the plate for 1-4 hours in the incubator.
4. Measure the absorbance at 450 nm using a microplate reader.
Note: If measuring the absorbance later, add 10 μ L of 0.1 M HCl solution or 1% w/v SDS solution to each well, and cover the culture plate to avoid light before storing at room temperature. No change should be observed in the absorbance value if measured within 24 hours.

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Cell proliferation toxicity test

1. Prepare 100 μL of cell suspension in a 96-well plate (5000 cells/well). Pre-culture the culture plate in the incubator for 24 h (37°C, 5% CO₂).
2. Add 1-10 μL of various concentrations of samples to be tested to the culture plate.
3. Incubate the culture plate in the incubator for an appropriate length of time (e.g., 6, 12, 24, or 48 h).
4. Add 10 μL of CCK-8 solution to each well.
Note: Be careful not to generate bubbles in the well, bubbles affect the OD reading.
5. Incubate the culture plate for 1-4 h in the incubator.

6. Measure the absorbance at 450 nm with a microplate reader.

Note: If measuring the absorbance later: add 10 μL of 0.1 M HCl solution or 1% w/v SDS solution to each well, and cover the culture plate to avoid light before storing at room temperature. No change should be observed in the absorbance value if measured within 24 hours.

Note: If the substance to be tested has oxidizing or reducing properties, you should replace the fresh medium before adding CCK-8 (remove the medium, wash the cells twice with the medium, and then add the new medium) to remove the influence of the substance.