Cell Counting Kit-3D (CCK-3D)

Introduction

Traditional cell culture is mainly based on 2D culture. With the deepening of cell research, scientists have found that 2D cultured cells are significantly different from cells under physiological conditions in vivo. In order to better simulate the growth of cells in the body, scientists are increasingly using 3D cell culture technology.

Cell Counting Kit-3D (CCK-3D) is convenient and sensitive for detecting cell proliferation/toxicity of 3D cells. It uses a reaction substrate of the WST series, which can be reduced by the abundant dehydrogenase in living cells in the presence of electron-coupled reagents and converted to the soluble, orange formazan in culture media. The amount of formazan produced by dehydrogenase is directly and linearly related to the number of viable cells.

This reagent is optimized for viability assays of a variety of cultured 3D spheroids, such as drop plates, ultra-low adsorption plates, Matrigel-coated and agarose-coated 3D spheroids. At the same time, this reagent is ready to use, making it ideal for high-throughput screenings.

Components and Storage

Components	K2270-100 T	K2270-500 T	K2270-1000 T	K2270-3000 T	K2270-10000 T
Cell Counting Kit-3D (CCK-3D)	1×1 mL	5×1 mL	2×5 mL	6×5 mL	20×5 mL
Store at -20°C away from light, stable for 1 year. For frequent use, keep it at 4°C away from light.					

Protocol

1. **Cell seeding:** For cell proliferation experiments, seed 100 μL of cell suspension (approximately 2000 cells/well) per well in a 96-well plate; For cytotoxicity experiments, seed 100 μL of cell suspension (approximately 5000 cells/well) per well in a 96-well plate. At the same time, it is necessary to set up a blank group (containing only medium).

***Note:** The amount of cell seeding varies depending on the cell types.

2. **Drug treatment (optional):** Treat cells with the interested drug for a certain time.
3. Add CCK-3D with 1/10 volume of medium per well. For 96-well plates, 10 μL of CCK-3D is needed per 100 μL medium.

***Note:** Be careful not to introduce bubbles into the wells, as they can interfere with detection.

4. Place the plate in the incubator and incubate for 0.5-3 h.

*Note: For most cases, incubation for 1 h is sufficient. The incubation time can be adjusted according to the specific experiment.

5. **Detection:** Gently pat the 96-well plate so that the orange-yellow product is evenly distributed within the wells. Subsequently, measure the absorbance at 450 nm using a microplate reader. If required, a wavelength greater than 600 nm, such as 650 nm, can also be used as a reference wavelength for dual-wavelength determination.

6. **Data analysis**

   Cell viability (%) = [(As-Ab) / (Ac-Ab)] X 100%

   Inhibition rate (%) = [(Ac-As) / (Ac-Ab)] X 100%

   As = Absorbance of experiment group (absorbance of wells containing cells, medium, CCK-3D, and tested drug)

   Ab = Absorbance of blank group (absorbance of wells containing medium and CCK-3D)

   Ac = Absorbance of control group (absorbance of wells containing cells, medium, and CCK-3D)

Note

1. Repeated freeze-thaw of this reagent will affect the detection effect. Although repeated freeze-thaw 3 times will not significantly affect the detection effect, it is recommended to avoid repeated freeze-thaw as much as possible. A small amount of precipitate may occur after thawing, which should be dissolved as much as possible by equilibrating this reagent to room temperature. For short-term frequent use, store this reagent at 4°C.
2. CCK-3D may react with reducing agents, which can interfere with detection if a reducing agent (e.g., some antioxidants) is used. The reducing agent needs to be removed before adding CCK-3D.
3. Care about not introducing bubbles to the wells, since they interfere with the O.D. reading.
4. The absorption value of phenol red in a culture medium can be removed by subtracting the absorption value of the blank group.
5. The toxicity of CCK-3D is very low, and after the completion of the CCK-3D assay, the cells can be used for other cell assays. However, in order to avoid the possible impact of CCK-3D on subsequent experiments, it is not recommended for other assays unless cells are extremely scarce.
6. Cells can be cultured in ultra-low adsorption plates designed for 3D culture and then transferred to common multi-well plates for detection.
7. We recommend inoculating the cells near the center of the plate, culture medium in the most peripheral wells of the plate is easy to evaporate, you can fill these wells with PBS or water.
8. For your safety and health, please wear lab coats and gloves during the experiment.

9. For research use only. Not to be used in clinical diagnostic or clinical trials.

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