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Cell Counting Kit

Product Number: F046

Shipping and Storage

Wet ice transportation; Valid for 12 months at 2-8℃; Long term use can also be stored at -20℃, valid for 24 months; Long term storage is recommended to avoid light exposure.

Component

Component	F046
Cell Counting Kit	1mL

Description

Cell Counting Kit, It is a rapid and highly sensitive detection kit widely used for cell proliferation and cytotoxicity. The detection principle is that in the presence of an electron coupling reagent, the WST-8 compound in the detection reagent is reduced by dehydrogenase in living cells to a water-soluble orange yellow formamide compound, which has a maximum absorption peak at 450 nm. The amount of formaldehyde is directly proportional to the number of live cells, that is, the more and faster the cell proliferation, the darker the color; The greater the cytotoxicity, the lighter the color. For the same cells, there is a linear relationship between the depth of color and the number of cells. WST-8 in this kit has no significant toxicity to cells and can be used for cell proliferation detection induced by exogenous cytokines, as well as cell toxicity detection induced by cytotoxic agents such as drugs, or cell growth inhibition detection induced by some drugs. It is an upgraded version of the MTT detection method.

This reagent kit is easy to use, does not require preparation or dilution, does not require cell collection or washing, and does not require re dissolution of methyl compounds. It is suitable for both adherent cells and suspended cells. And phenol red and serum have no significant effect on the determination of this kit.

Protocol

1. Create a standard curve (perform this operation when determining the specific number of cells)

1.1. First, count the number of cells in the prepared cell suspension using a cell counting plate, and then inoculate the cells.

1.2. Dilute the culture medium proportionally to form a cell concentration gradient. Generally, 3-5 cell concentration gradients are required, with 3-6 replicates per group.

1.3. After inoculation, culture the cells until they adhere to the wall, then add CCK reagent to culture for a certain period of time and measure the OD value. Create a standard curve with cell number as the x-axis and OD value as the y-axis. According to this standard curve, the number of cells in unknown samples can be determined (the premise of using this standard curve is that the experimental conditions must be consistent to determine the number of cells inoculated and the culture time after adding CCK).

2. Cell viability testing

2.1. Inoculate cell suspension (100μL/well) into a 96 well plate. Place the culture plate in the incubator for pre cultivation (at 37℃, 5% CO_2).

2.2. Add 10μL of CCK solution to each well (be careful not to generate bubbles in the well, as they can affect the OD reading).

2.3. Incubate the culture plate in the incubator for 2 hours.

2.4. Measure the absorbance at 450nm using an enzyme-linked immunosorbent assay (ELISA) reader.

2.5. If the OD value is not measured temporarily and plans to be measured in the future, 10μL of 0.1M HCl or 1% SDS (W/V) solution can be added to each well, and the culture plate can be covered and stored at room temperature in the dark. The absorbance will not change within 24 hours.

3. Cell proliferation toxicity detection

3.1. Prepare 100μL of cell suspension in a 96 well plate. Pre culture the culture plate in the incubator for 24 hours (at 37℃, 5% CO_2).

3.2. Add 10μL of the test substance at different concentrations to the culture plate. Incubate in the incubator for an appropriate period of time (e.g. 6, 12, 24, or 48 hours).

3.3. Add 10μL of CCK solution to each well (be careful not to generate bubbles in the well, as they can affect the OD reading). If the substance to be tested has oxidizing or reducing properties, fresh culture medium can be replaced before adding CCK (remove the culture medium, wash the cells twice with the culture medium, and then add new culture medium) to remove drug effects.

3.4. Incubate the culture plate in the incubator for 2 hours.

3.5. Measure the absorbance at 450nm using an enzyme-linked immunosorbent assay (ELISA) reader.

3.6. If the OD value is not measured temporarily and plans to be measured in the future, 10μL of 0.1M HCl or 1% SDS (W/V) solution can be added to each well, and the culture plate can be covered and stored at room temperature in the dark. The absorbance will not change within 24 hours.

4. Vitality calculation

Cell viability (%)=[A (dosing) - A (blank)]/[A (0 dosing) - A (blank)] × 100

A (Medication): Absorbance of pores containing cells, CCK solution, and drug solution

A (blank): Absorbance of wells with culture medium and CCK solution without cells

A (0 dosing): absorbance of wells with cells and CCK solution but without drug solution

Cell viability: Cell proliferation viability or cytotoxic viability

Note

1. The detection principle of this kit relies on the reaction catalyzed by dehydrogenase. If there are too many reducing agents (such as some antioxidants) in the sample, they will interfere with the detection and need to be removed.
2. Suggest making a few wells first to explore the number of cells to be inoculated and the cultivation time after adding CCK reagent.
3. White blood cells may need to be cultured for a longer period of time.
4. When using a standard 96 well plate, the minimum inoculation amount of adherent cells should be at least 1000 cells per well (100μL culture medium). The sensitivity of detecting white blood cells is relatively low, so it is recommended to inoculate no less than 2500 cells per well (100μL culture medium). If you want to use a 24 well or 6-well plate for the experiment, please first calculate the corresponding inoculation amount for each well, and add CCK solution at 10% of the total volume of the culture medium for each well.
5. If there is no 450nm filter, a filter with absorbance between 430-490nm can be used, but 450nm has the highest detection sensitivity.
6. The absorbance of phenol red in the culture medium can be eliminated by subtracting the absorbance of the background in the blank well during calculation, so it will not affect the detection.
7. Avoid generating bubbles when adding detection reagents, otherwise it may interfere with the detection results.
8. For your safety and health, please wear lab coats and disposable gloves when operating.