Product Information

Catalog number: REK0002

Size: 100T/500T/1000T

Storage: CCK-8 can be stored at 0-5℃ away from light for 12 months, and can be stored for longer at -20℃. Repeated thawing and freezing will increase the background value and interfere with the experimental determination. If you need to use it frequently, please store the reagent at 0-5℃.

Product Description

CCK-8 test kit is a highly sensitive, non-radioactive colorimetric assay for determining the number of live cells in cell proliferation or toxicity experiments.

CCK-8 solution can be added directly to cell samples without pre-mixing various components. It is fast to detect and has very low toxicity. CCK-8 is based on the water-soluble tetrazolium salt. WST8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt]. The working principle is: WST8 can be reduced by dehydrogenase in mitochondria to generate orange-yellow formazan in the presence of electron coupling reagents. Formazan dye can be dissolved in tissue culture medium and is proportional to the number of live cells. Through colorimetry, the number of live cells can be dynamically quantified, thereby detecting cell proliferation or drug toxicity.

Cautions

1. For the first experiment, it is recommended to use a few wells to explore the number of cells to be inoculated and the culture time after adding CCK-8 reagent.
2. When inoculating, pay attention to mixing the cell suspension to avoid cell sedimentation, resulting in unequal numbers of cells in each well. It is recommended to mix every few wells inoculated. The culture medium in the circle of wells around the culture plate is easy to evaporate. In order to reduce errors, it is recommended to add only culture medium to each well on the four sides of the culture plate, and not to use it as an indicator detection well.
3. The culture time varies depending on the type of cells and the number of cells in each well. In general, white blood cells are more difficult to color, so a longer reaction time or an increase in the number of cells (~10^5cells/well) is required. Suspension cells are more difficult to color than adherent cells. For suspension cells, remove them from the incubator after adding CCK-8 for 1-4 hours and determine the degree of staining by visual inspection or using an enzyme marker. If color development is difficult, the culture time can be extended and tested again; for adherent cells, the culture time of CCK-8 is generally 1-4 hours, but the color development can be observed with the naked eye after about 30 minutes of culture (depending on the cell type,

you need to explore the conditions).

4. It is recommended to use a multi-channel pipette to reduce the difference between parallel wells. When adding CCK-8 reagent, it is recommended to add it obliquely against the wall of the culture plate, and do not insert it below the surface of the culture medium to add it, which is easy to produce bubbles and interfere with the OD value reading.
5. When adding CCK-8 reagent, the speed should be fast to reduce the residue of reagent on the pipette. In order to fully mix the CCK-8 reagent and the culture medium, it is recommended to gently shake the culture plate after adding the reagent. In order to avoid the error caused by the residue of CCK-8 reagent on the tip of the gun when adding the sample, the CCK-8 reagent can be diluted with the culture medium before adding the sample and mixed before adding the sample.
6. WST8 in CCK-8 reagent will react with reducing agent to generate WST8 formazan. If there is reducing agent in the experiment, please check the background OD value, that is, add drugs to the culture medium without cells, then add CCK-8 reagent for a certain period of time, and compare it with the culture medium without drugs (only CCK-8 reagent). If the OD value is obviously higher, it means there is a reaction.
7. If the cell culture time is long, the color of the culture medium changes or the pH changes, it is recommended to replace the fresh culture medium before adding CCK-8 reagent. The culture medium containing phenol red does not affect the determination of cell activity by this kit.
8. If the sample is a highly turbid cell suspension, it is recommended to set 600 nm (or above 600 nm) as the reference wavelength and deduct the OD value of the reference wavelength.
9. CCK-8 reagent has very low toxicity to cells. It continuously reacts with the dehydrogenase in living cells to make the solution color darker and the OD value increase (Note: the dehydrogenase in living cells is continuously produced). In addition, other experiments such as neutral red method or crystal violet method can also be continued after the CCK-8 method is completed.
10. If you want to determine the specific number of cells, it is recommended to make a standard curve first.

Instructions

Make a standard curve

1. Count the number of cells in the prepared cell suspension with a cell counting plate, and then inoculate the cells.
2. Dilute the cells in the culture medium in equal proportions (e.g. 1/2 ratio) to form a cell concentration gradient. Generally, 3-5 cell concentration gradients should be made, with 3-6 duplicate wells in each group.
3. After inoculation, culture the cells for 2-4 hours to allow them to adhere to the wall, then add CCK-8 reagent and culture for a certain period of time to measure the OD value, and make a standard curve with the number of cells as the horizontal axis (X axis) and the OD value as the vertical axis (Y axis). Based on this standard curve, the number of cells in unknown samples can be determined (the prerequisite for using this standard curve is that the experimental conditions must be consistent, which is convenient for determining the number of cells to be inoculated and the culture time after adding CCK-8).

Calculation

Cell survival rate = [(As-Ab] / (Ac-Ab)] × 100%

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

As: experimental well (culture medium containing cells, CCK8, toxic substances)

Ac: control well (culture medium containing cells, CCK8, no toxic substances)

Ab: blank well (culture medium without cells and toxic substances, CCK8)

Cell Viability Assay

1. Inoculate the cell suspension (100μl/well) in a 96-well plate. Pre-culture the plate in an incubator (at 37°C, 5% CO_2).
2. Add 10ul of CCK-8 solution to each well (be careful not to create bubbles in the wells, as they will affect the OD reading).
3. Incubate the plate in the incubator for 1-4 hours.
4. Measure the absorbance at 450nm using an ELISA reader.
5. If you do not measure the OD value for the time being and plan to measure it later, add 10μl of 0.1 M HCl solution or 1% w/v SDS solution to each well and cover the plate to avoid light and store at room temperature. The absorbance will not change within 24 hours.

Cell Proliferation and Cytotoxicity Assay

1. Prepare 100 μl of cell suspension in a 96-well plate. Pre-incubate the plate in an incubator for 24 hours (at 37°C, 5% CO_2).
2. Add 10 μl of the test substance at different concentrations to the plate.
3. Incubate the plate in an incubator for an appropriate period of time (e.g. 6, 12, 24 or 48 hours).
4. Add 10 μl of CCK-8 solution to each well (be careful not to create bubbles in the wells, as they will affect the OD reading).
5. Incubate the plate in an incubator for 1-4 hours.
6. Measure the absorbance at 450 nm using a microplate reader.

Note: If you are not measuring the OD value for the time being and plan to measure it later, you can add 10 μl of 0.1 M HCl solution or 1% w/v SDS solution to each well and cover the plate and store it at room temperature in dark. The absorbance will not change within 24 hours. Note: If the substance to be tested is oxidizing or reducing, fresh culture medium can be replaced before adding CCK-8 to remove the influence of the drug. Of course, if the influence of the drug is relatively small, the culture medium can be left unchanged, and the blank absorption after adding the drug to the culture medium can be directly deducted.

Notes

This product is for research use only.