Cell Counting Kit Instructions

Cat No:IV08

I. Making standard curve (measuring cell specific number)

1. Count the number of cells in the cell suspension prepared by using a cell count plate and then inoculate cells.
2. It is generally consider that a gradient of 3-5 cell concentration is done by that ratio of the culture medium to the concentration gradient of one cell by proportion (for example: 1/2 ratio). 3-6 multiple Wells are use for each group.
3. After inoculation, the cell adhered to the wall for 2 to 4 hours after inoculation, and then CCK reagent was added to determine the OD value after a certain time, and a standard curve was produced with the number of cells as the X-axis (X-axis) and the OD value as the Y-axis (Y-axis). According to the standard curve, we can determine the number of cells in the unknown sample.

II. Cell activity detection

1. Inoculate cell suspension (100 mu; L/pore) in 96 well plate. Culture methods (keep the culture plate in the incubator at 37 ℃ and 5% CO2 conditions).
2. .Add 10uL of CCK solution to each port (take care not to generate bubbles in the hole, which will affect the reading of the OD value).
3. Incubate the plate within the incubator for 1-4 hours.
4. Measure the absorbance at 450nm with an enzyme marker.
5. If the OD value is not determined for the time being, if it is determined to be determined in the future, it can be added to the HCL solution or 1% w/v SDS solution of 10 mu L 0.1M in each hole, and cover the culture plate to avoid light and keep it at room temperature. Absorbance within 24 hours will not change.

III. Cell proliferation -Cytotoxicity detection

1. 100 mul of cell suspension in 96 well plate. The participation in the incubator culture plate 24 hours under the condition of this (at 37 ℃ and 5% CO2) .
2. Add a different concentration of the substance to be tested to the culture plate.
3. Incubate the culture plate in the incubator for a suitable period of time (e.g. 6, 12, 24 or 48 hours).
4. Add 10 to each hole (Be careful not to generate bubbles in the hole, it will affect the reading of OD value).
5. Incubate the plate within the incubator for 1-4 hours.
6. Measure the absorbance at 450nm with an enzyme marker.
7. If the OD value is not determined tentatively, it is possible to add 10 mul of 0.1 M HCL solution or 1 % w/v SDS solution to each hole and cover the panel shelter at room temperature. Absorbance within 24 hours will not change.

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Note:*If you have any oxidizing or reducing the material under test, and can be replaced before adding CCK fresh medium (removal of medium and culture medium washing cells twice, then add the new medium), get rid of drugs. Of course, if the influence of the drug is small, the culture medium may not be changed, and the medium may be directly deducted for the blank absorption after the medicine is added.*

Background

CCK is a one-bottle solution;no premixing of components is required.CCK ,being nonradioactive, allows sensitive,Cell viability and cytotoxicity assays are used for drug screening and cytotoxicity tests of chemicals.Since WST-8 formazan is water soluble, it does not form crystals. Therefore, solubilizing process such as MTT assay is not required. Additionally, The detection sensitivity of CCK-8 is higher than the other tetrazolium salts such as MTT, XTT, MTS or WST-1,colorimetric assays for the determination of the number of viable cells in cell proliferation and cytotoxicity assays.