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Datasheet

Ver.1 Date : 20180222

CCK-8 Cell Counting Kit

Cat# CC1007 – 500 rxn / CC1008 – 1000 rxn

CCK-8 is stable at 4°C with protection from light. Store it at -20°C for longer storage.

INTRODUCTION

CCK-8 Cell Counting Kit is based on CCK8 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium,monosodium salt] which is rapid, highly sensitive and widely used in detection of cell proliferation and cytotoxicity. CCK8, similar to MTT, produces a water-soluble formazan dye upon reduction in the presence of an electron carrier by dehydrogenases in mitochondria. The amount of the formazan dye generated by the activity of dehydrogenases in cells is directly proportional to the number of living cells. CCK-8 owns obvious advantages compared with other tetrazolium salts such as MTT, XTT, MTS or WST-1. Dehydrogenases in mitochondria reducing the tetrazolium dye MTT to its insoluble formazan which need to be solubled by special solvent. But CCK-8 is a one-bottle solution and no solubilization stepsare required. Compared with MTT and XTT, the detection sensitivity using CCK-8 is higher and the linear is wider. The fact that Phenol red and serum do not have significantly effect on determination of the kit has been confirmed. One-bottle, ready-to-use solution of CCK8 is in the kit. The kit is suitable for determination of large quantities of samples and directly added to cell supernatant and incubated for some time. After adding color, it can be used to read the plate repeatedly in different time, so that the detection time is more flexible and easy to draw the growth curve.

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No	Component	**CC1007 – 500 rxn**	**CC1008 – 1000 rxn**
AA	CCK8 solution	5 ml	10 ml

PROTOCOL

1. Dispense 100 µl of cell suspension (about 5000-10000 cells/well) in a 96-well plate filled the edged wells with Sterile water or PBS. Set blank wells (There is no cell but with normalculture medium) and control (no drug).Set 3-5 repeat well in each group.
2. Pre-incubate the plate for 24 hours in a humidified incubator (e.g., at 37°C, 5% CO2).Observe under an inverted microscope.
3. Add 10 µl of various concentrations of substances to be tested to the plate and incubate at 37°C.
4. Add 10 µl of CCK-8 solution to each well of the plate and incubate at 37°C for 1 - 4 hours.
5. Measure the absorbance at 450 nm using a microplate reader. If there are no filters with 450nm, the absorbance between 450 and 490 nm is also appropriate.
6. Results analyze. The OD value of tested well minus OD value of blank or control. Take average of the OD value of each repetition well.

Cell viability % = (Drug OD- Blank OD/ Control OD- Blank OD) x 100%

Note :

1. If store at -20°C, need to avoid repeated thawing and freezing since repeated thawing and freezing causes an increase in the background, which interferes with the assay. Recommended small dose packing and keep the kit from light with dark or black foil bag.
2. Prepare the tested drug with culture medium or PBS. If the drug has reducibility only determines the OD value of the tested drug solution containing CCK-8 with no cells. If the OD value is small, just directly add CCK8 to the cell. In contrast, remove the culture medium and wash the cells twice with fresh medium. Before the determination, add 100 μl fresh medium and 10 μl CCK8 to the cells.
3. Try to avoid destroying cells; manipulation to the cells should be careful.
4. The incubated time with CCK8 is dependent on the type and number of cells. One hour is enough for the most of cells but for leukocyte time need to be longer.
5. If 96-well plate is not used for this assay, please calculate the number of cells per well accordingly.

PRODUCT USE LIMITATION

These products are intended for research use only.