V02

CCK-8 试剂盒(细胞增殖及毒性检测试剂盒)

货号：CA1210
规格：100T/500T/1000T
保存：2-8℃，避光保存，有效期 1 年。

产品组成：

名称	100T	500T	1000T
CCK-8 溶液	1mL	5mL	10mL

产品介绍：

CCK-8 试剂盒（Cell Counting Kit-8）是一种基于 WST-8 的快速、高灵敏度细胞活性检测的试剂盒，广泛应用于细胞增殖和细胞毒性的检测。WST-8 是一种类似于 MTT 的化合物，在电子耦合试剂存在的情况下，可以被线粒体内的一些脱氢酶还原生成橙黄色的水溶性甲臜化合物。细胞增殖越多越快，则颜色越深；细胞毒性越大，则颜色越浅。对于同种类型细胞，颜色深浅与细胞数量在一定限度内呈线性关系。

本试剂盒为即用型溶液，无需再行配制，可直接用于 96 孔板细胞实验，省略了细胞洗涤与收集等步骤。该试剂盒可用于细胞因子等诱导的细胞增殖检测，也可用于抗癌药物等对细胞有毒试剂诱导的细胞毒性检测，或一些药物诱导的细胞生长抑制检测。

操作步骤：（*仅供参考*）

一、制作标准曲线

1. 先用细胞计数板计数所制备的细胞悬液中的细胞数量，然后接种细胞。
2. 按比例依次用培养基等比稀释成一个细胞浓度梯度，一般要做 3-5 个细胞浓度梯度，每组 3-6 个复孔。
3. 接种后培养至细胞贴壁，然后加 CCK-8 溶液培养一定时间后测定 OD 值，制作出一条以细胞数量为横坐标（X 轴），OD 值为纵坐标（Y 轴）的标准曲线。
4. 根据此标准曲线可以测定出未知样品的细胞数量（试用此标准曲线的前提是实验的条件要一致，便于确定细胞的接种数量以及加入 CCK-8 后的培养时间。）

二、细胞活性检测

1. 在 96 孔板中接种细胞悬液（100μL/孔）。将培养板放在培养箱中预培养（在 37℃，5% CO2 的条件下）。
2. 向每孔加入 10μL CCK-8 溶液（注意不要在孔中生成气泡，它们会影响 OD 值的读数）。
3. 将培养板在培养箱内孵育 1-4 小时。
4. 用酶标仪测定在 450nm 处的吸光度。
5. 如果暂时不测定 OD 值，打算以后测定的话，可以向每孔中加入 10μL 0.1M 的 HCl 或者 1%SDS(W/V)溶液，并遮盖培养板在室温条件下避光保存。在 24 小时内吸光度不会发生变化。

三、细胞增值-毒性检测

1. 在 96 孔板中配置 100μL 的细胞悬液。将培养板置于培养箱预培养 24 小时（在 37 ℃，5% CO_2 的条件下）。
2. 向培养板加入 10μL 不同浓度的待测物质。在培养箱孵育一段时间（例如：6、12、24 或 48 小时）。
3. 向每孔加入 10μL CCK-8 溶液（注意不要在孔中生成气泡，它们会影响 OD 值的读数）。如果待测物质有氧化性或还原性的话，可在加 CCK-8 溶液之前更换新鲜培养基（除去培养基，并用培养基洗涤细胞两次，然后加入新的培养基），去掉药物影响。
4. 将培养板在培养箱内孵育 1-4 小时，用酶标仪测定在 450nm 处的吸光度。
5. 如果暂时不测定 OD 值，可以向每孔中加入 10μL 0.1M 的 HCl 或者 1%SDS(W/V)溶液，并遮盖培养板避光保存在室温条件下。在 24 小时内吸光度不会发生变化。

活力计算：

细胞活力（%）=[A(加药)－A(空白)] / [A(0 加药)－A(空白)]×100
A（加药）：具有细胞、CCK-8 溶液和药物溶液的孔的吸光度
A（空白）：具有培养基和 CCK-8 溶液而没有细胞的孔的吸光度
A（0 加药）：具有细胞、CCK-8 溶液而没有药物溶液的孔的吸光度

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细胞活力：细胞增殖活力或细胞毒性活力

注意事项：

1. 本试剂颜色应为粉红色，如果发现溶液变黄应弃用。
2. 由于使用 96 孔板进行检测，如果细胞培养时间较长，一定要注意蒸发问题。由于 96 孔板周围一圈最容易蒸发，可以采取弃用周围一圈的办法，改加相同量的 PBS、水或培养液。
3. 本试剂盒的检测依赖于脱氢酶催化的反应，所以还原剂(例如一些抗氧化剂)会干扰检测，如果待检测体系中存在较多的还原剂，需设法去除。
4. 建议先做几个孔摸索接种细胞的数量和加入 CCK-8 试剂后的培养时间，白细胞可能培养时间较长。
5. 当使用标准 96 孔板时，贴壁细胞的最小接种量至少为 1000 个/孔 (100μL 培养基)。检测白细胞时的灵敏度相对较低，因此推荐接种量不低于 2500 个/孔 (100μL 培养基)。如果要使用 24 孔板或 6 孔板实验，请先计算每孔相应的接种量，并按照每孔培养基总体积的 10%加入 CCK-8 溶液。
6. 如果没有 450nm 的滤光片，可以使用吸光度在 430-490nm 之间的滤光片，但是 450nm 检测灵敏度最高。
7. 培养基中酚红的吸光度可以在计算时，通过扣除空白孔中本底的吸光度而消去，因此不会对检测造成影响。

V02

Cell Counting Kit-8

Cat: CA1210
Size: 100T/500T/1000T
Storage: 2-8℃, avoid light, valid for 1 year。

Kit Components

Reagent	100T	500T	1000T
Cell Counting Kit-8	1mL	5mL	10mL

Introduction

The Cell Counting Kit-8(CCK-8 Kit) is a fast and highly sensitive cell activity detection kit based on WST-8, widely used for cell proliferation and cytotoxicity detection. WST-8 is a compound similar to MTT that can be reduced by some dehydrogenases in mitochondria in the presence of electron coupling reagents to produce orange yellow water-soluble formaldehydes. The more cells proliferate, the faster the color becomes; The greater the cytotoxicity, the lighter the color. For the same type of cells, there is a linear relationship between the color depth and the number of cells within a certain limit.

This kit is a ready-to-use solution that does not require further preparation and can be directly used for 96 well plate cell experiments, omitting steps such as cell washing and collection. This kit can be used for cell proliferation detection induced by cytokines, cytotoxicity detection induced by cytotoxic agents such as anticancer drugs, or cell growth inhibition detection induced by some drugs.

Protocol(*for reference only*)

Making standard curves

1. First, count the number of cells in the prepared cell suspension using a cell counting chamber, and then inoculate the cells.
2. Dilute the culture medium proportionally in order to form a cell concentration gradient. Generally, 3-5 cell concentration gradients are required, with 3-6 wells in each group for average value.
3. After inoculation, culture until the cells adhere to the wall, and then add Cell Counting Kit-8 to culture for a certain time. Measure the OD value, and create a standard curve with the number of cells as the horizontal axis (X-axis) and the OD value as the vertical axis (Y-axis).
4. According to this standard curve, the number of cells in unknown samples can be determined (the premise for using this standard curve is that the experimental conditions must be consistent, which is convenient for determining the number of cells inoculated and the cultivation time after adding Cell Counting Kit-8.)

Cell activity detection

1. Inoculate cell suspension (100μL/well) in a 96 well plate. Place the culture plate in an incubator for pre cultivation (at 37 ℃, 5% CO2).
2. Add 10μL of Cell Counting Kit-8 to each well (be careful not to generate bubbles in the well as they may affect the reading of the OD value).
3. Incubate the culture plate in the incubator for 1-4 hours.
4. Measure the absorbance at 450nm using an enzyme-linked immunosorbent assay.
5. If the OD value is not measured temporarily, you can add 10μL of 0.1M HCl or 1% SDS (W/V) solution to each well, and the culture plate can be covered and stored in dark at room temperature. The absorbance will not change within 24 hours.

Cell proliferation toxicity detection

1. Prepare 100μL of cell suspension in a 96 well plate. Pre cultivation the culture plate in the incubator for 24 hours (at 37 ℃, 5% CO2).
2. Add 10μL of different concentrations of the substance to be tested to the culture plate. Incubate in the incubator for a period of time (e.g. 6, 12, 24, or 48 hours).
3. Add 10μL of Cell Counting Kit-8 to each well (be careful not to generate bubbles in the well as they may affect the reading of the OD value). If the substance to be tested has oxidizing or reducing properties, fresh culture medium can be replaced before adding Cell Counting Kit-8 (remove the culture medium, wash the cells twice with the culture medium, and then add a new culture medium) to remove the drug effect.
4. Incubate the culture plate in the incubator for 1-4 hours, and measure the absorbance at 450nm using an enzyme-linked immunosorbent assay.

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

Vitality calculation

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

A(dosing)：Absorbance of wells with cells, Cell Counting Kit-8, and drug solution

A(blank)：Absorbance of wells with culture medium and Cell Counting Kit-8 without cells

A(0 dosing)：Absorbance of wells with cells and Cell Counting Kit-8 without drug solution

Cell viability:cell proliferation activity or cell cytotoxic activity

Note

1. The color of this reagent should be pink, and if the solution turns yellow, it should be discarded.
2. Due to the use of 96 well plates for detection, it is important to pay attention to evaporation issues because of long culture time . Due to the fact that the area around the 96 well plate is most prone to evaporation, can discard the surrounding area and add the same amount of PBS, water, or culture medium instead of cells.
3. The kit relies on dehydrogenase-catalyzed reactions, so reducing agents (such as some antioxidants) may interfere with the detection. If there are many reducing agents in the system to be tested, it is necessary to try to remove them.
4. It is recommended to first make a few holes to explore the number of inoculated cells and the cultivation time after adding Cell Counting Kit-8. White blood cells may take longer to cultivate.
5. When using a standard 96 well plate, the minimum inoculation amount for adherent cells is 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 at least 2500 cells per well (100μL culture medium). If you want to use a 24 well or 6 well plate experiment, please first calculate the corresponding inoculation amount for each well and add Cell Counting Kit-8 at 10% of the total volume of culture medium for each well.
6. If there is no 450nm filter, a filter with an absorbance between 430-490nm can be used, but 450nm has the highest detection sensitivity.
7. 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.