

# Cell Counting Kit-8 (CCK-8)

# 细胞增殖-毒性检测试剂盒

(100孔, 500孔, 1,000孔, 3,000孔以及10,000孔的规格)

## 注意事项:

- 1 第一次做实验时, 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。
- 2 接种时注意细胞悬液一定要混匀, 以避免细胞沉淀下来, 导致每孔中的细胞数量不等, 可以每接种几个孔就混匀一下。培养板周围一圈孔培养基容易挥发, 为了减少误差, 建议培养板的四边每孔只加培养基, 而不作为指标检测孔。
- 3 培养时间根据细胞种类的不同和每孔内细胞数量的多少而异。一般情况下, 白细胞较难显色, 因此需要较长的CCK-8反应时间或增加细胞数量 ( $\sim 10^5$  个细胞/孔)。悬浮细胞与贴壁细胞相比较难显色。对于悬浮细胞, 在加入CCK-8培养1-4小时后, 可先从培养箱中取出, 目测染色程度或用酶标仪测定决定。若显色困难, 可将培养板放回培养箱, 继续培养数小时后再确定。对于贴壁细胞, CCK-8的培养时间一般为1-4小时, 但在培养30分钟左右即可取出肉眼观察显色程度 (根据细胞种类而定, 需要摸索一下条件)。注意: CCK-8的最佳反应时间以具体显色的最佳时间为准。
- 4 有条件的情况下建议采用多通道移液器, 可以减少平行孔间的差异。加 CCK-8试剂时, 建议斜贴着培养板壁加, 不要插到培养基液面下加, 容易产生气泡, 会干扰O.D值读数。
- 5 加 CCK-8试剂时速度要快, 减少试剂在移液器上的残留。为使CCK-8试剂和培养基充分混匀, 建议在加入CCK-8试剂后轻轻振摇培养板。为了避免加样时由于CCK-8试剂在枪头上的残留所带来的误差, 可以在加样前用培养基稀释CCK-8试剂并混匀后加样。
- 6 CCK-8试剂中的WST<sup>®</sup>-8会与还原剂反应生成WST<sup>®</sup>-8甲臜, 如果实验中有还原剂, 请检查背景的O.D值, 即在不含细胞的培养基中加入药物, 然后加入CCK-8试剂在一定时间内检测, 和不加药物的培养基进行比较 (只加CCK-8试剂), 如果O.D值明显偏高, 则说明有反应。
- 7 若细胞培养时间较长, 培养基颜色发生变化或pH发生变化, 建议更换新鲜的培养基后再加CCK-8试剂。含有酚红的培养基不影响本试剂盒做细胞活性的测定。
- 8 如果样品为高浑浊度的细胞悬液, 建议设定600 nm (或600 nm以上) 作为参比波长, 扣除参比波长的O.D值即可。
- 9 CCK-8试剂对细胞的毒性非常低。它和活细胞内的脱氢酶持续反应使溶液颜色不断加深, O.D值不断增加 (注: 活细胞内的脱氢酶是持续产生的)。另外, 其他的实验, 例如中性红法或结晶紫法, 也可在CCK-8法检测完后继续进行。
- 10 如果要测定细胞的具体数量, 建议先做一个标准曲线 (具体方法参见P3的“制作标准曲线”)。

## 试剂内含

- 100 孔: 1 ml x 1 管
- 500 孔: 5 ml x 1 瓶
- 1,000 孔: 5 ml x 2 瓶
- 3,000 孔: 5 ml x 6 瓶
- 10,000 孔: 100 ml x 1 瓶

## 贮藏条件

CCK-8在避光0-5 °C的条件下可以存放2年。

## 所需设备和材料:

- 10  $\mu$ l、100-200  $\mu$ l以及多通道移液器
- 带有450 nm滤光片的酶标仪
- 96孔培养板
- CO<sub>2</sub>培养箱



## 概述

Cell Counting Kit-8 (CCK-8) 利用了Dojindo开发的水溶性四唑盐 — WST<sup>®</sup>-8 (2-(2-甲氧基-4-硝苯基)-3-(4-硝苯基)-5-(2,4-二硝基苯)-2H-四唑单钠盐)，它在电子载体1-Methoxy PMS存在的情况下能够被还原成水溶性的甲臞染料，如图1所示。

**WST<sup>®</sup>:** WST是同仁化学研究所的注册商标

CCK-8溶液可以直接加入到细胞样品中；不需要预配各种成分。CCK-8法是用于测定细胞增殖或毒性实验中活细胞数目的一种高灵敏度，无放射性的比色检测法。WST<sup>®</sup>-8被细胞内脱氢酶氧化还原后生成的橙黄色甲臞染料能够溶解在组织培养基中(如图2所示)，生成的甲臞量与活细胞数量成正比。

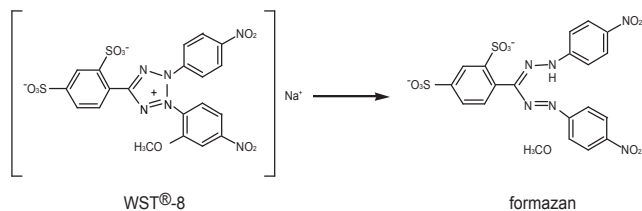


图1. WST<sup>®</sup>-8和WST<sup>®</sup>-8甲臞的结构

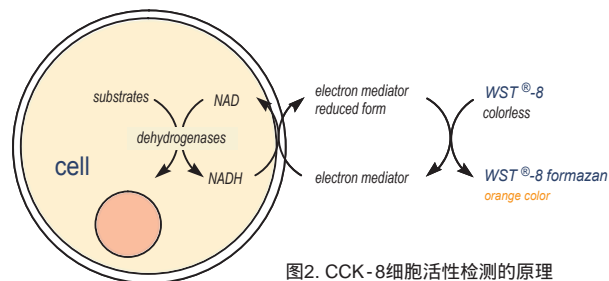


图2. CCK-8细胞活性检测的原理

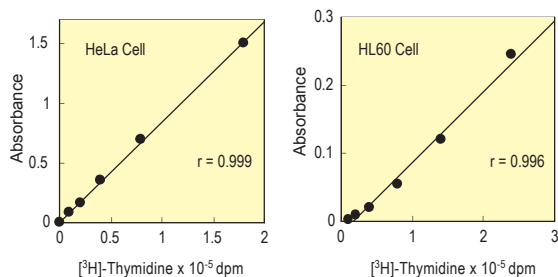


图3. CCK-8法与[3H]-thymidine掺入法之间的相关性

Medium: HeLa.....MEM, 10% FBS  
HL60.....RPMI1640, 10% FBS  
[3H]-Thymidine.....37 KBq/well  
Reagent: CCK-8.....10  $\mu$ l/well  
[3H]-Thymidine assay.....4hours  
Incubation: CCK-8.....3 hours

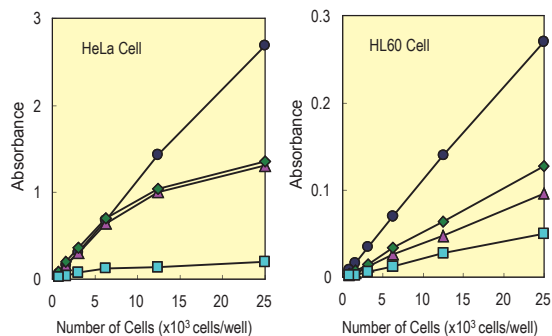
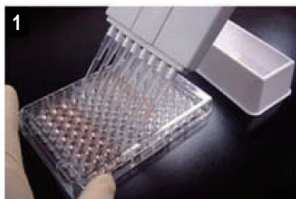


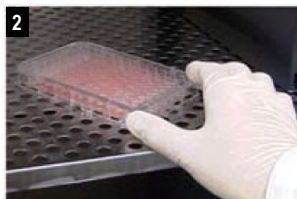
图4. 使用CCK-8与其他试剂灵敏度的比较

Medium: HeLa.....MEM, 10% FBS  
HL60.....RPMI1640, 10% FBS  
Incubation: HeLa.....37  $^{\circ}$ C, 5% CO<sub>2</sub>, 2 hours  
HL60.....37  $^{\circ}$ C, 5% CO<sub>2</sub>, 3 hours  
Detection: CCK-8 (●).....450 nm, XTT (◆).....450 nm  
MTS (▲).....490 nm, MTT (■).....570 nm

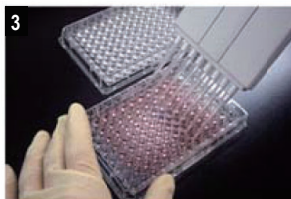
## 操作说明



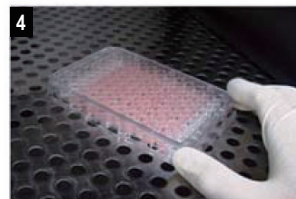
在96孔板中每孔加入100  $\mu$ l的细胞悬液



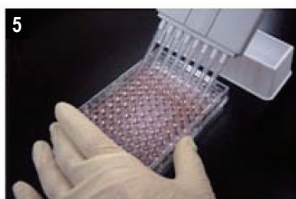
在培养箱中预培养细胞



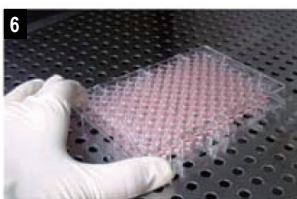
向培养板中加入药物(如果不加药物,可直接进行第5步操作)



在培养箱中培养一段时间



向每孔加入10  $\mu$ l CCK-8溶液



在培养箱中培养1-4小时(可根据自己的实验情况调整时间)



用酶标仪测定在450 nm处的吸光度

## 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 (例如：1/2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2-4小时使细胞贴壁，然后加CCK-8试剂培养一定时间后测定O.D值，制作出一条以细胞数量为横坐标 (X轴)，O.D值为纵坐标 (Y轴) 的标准曲线。根据此标准曲线可以测定出未知样品的细胞数量 (使用此标准曲线的前提条件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)。

\* 如果暂时不测定O.D值，打算以后测定或为了避免每次准备标准曲线，可以向每孔中加入10  $\mu$ l Stop Solution，并遮盖培养板避光保存在0-5条件下。在7天内吸光度不会发生变化。

## 细胞增殖—毒性检测

1. 在96孔板中配制100  $\mu$ l的细胞悬液。将培养板在培养箱预培养24小时 (在37℃, 5% CO<sub>2</sub>的条件下)。
2. 向培养板加入10  $\mu$ l不同浓度的待测物质。
3. 将培养板在培养箱培养一段适当的时间 (例如: 6, 12, 24或48小时)。
4. 向每孔加入10  $\mu$ l CCK-8溶液 (注意不要在孔中生成气泡, 它们会影响O.D值的读数)。
5. 将培养板在培养箱内培养1-4小时。
6. 用酶标仪测定在450 nm处的吸光度。

\* 如果暂时不测定O.D值，打算以后测定，可以向每孔中加入10  $\mu$ l Stop Solution，并遮盖培养板避光保存在0-5条件下，在7天内吸光度不会发生变化。

**注意：**如果待测物质有氧化性或还原性，可在加CCK-8之前更换新鲜培养基，去掉待测物质的影响。当然待测物质影响比较小的情况可以不更换培养基，直接扣除培养基中加入待测物质后的空白吸收即可。

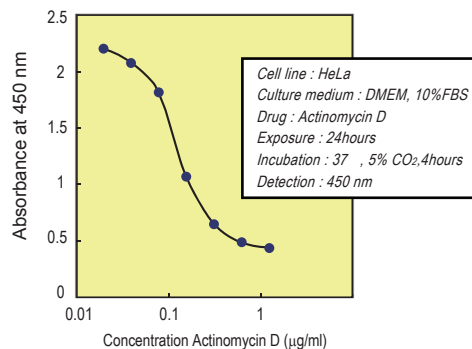


图5. 细胞毒性试验 (Actinomycin D)

计算公式：

$$\text{细胞存活率} = [(As - Ab) / (Ac - Ab)] \times 100\%$$

$$\text{抑制率} = [(Ac - As) / (Ac - Ab)] \times 100\%$$

As：实验孔 (含有细胞的培养基、CCK-8、待测物质)

Ac：对照孔 (含有细胞的培养基、CCK-8、没有待测物质)

Ab：空白孔 (不含细胞和待测物质的培养基、CCK-8)

## 细胞活性检测

1. 在96孔板中接种细胞悬液 (100  $\mu$ l / 孔)。将培养板放在培养箱预培养 (在37℃, 5% CO<sub>2</sub>的条件下)。
2. 向每孔加入10  $\mu$ l的CCK-8溶液 (注意不要在孔中生成气泡，它们会影响O.D值的读数)。
3. 将培养板在培养箱内培养1-4小时。
4. 用酶标仪测定在450 nm处的吸光度。

\* 如果暂时不测定O.D值，打算以后测定，可以向每孔中加入10  $\mu$ l Stop Solution，并遮盖培养板避光保存在0-5条件下，在7天内吸光度不会发生变化。

## 关联产品

应用	产品名称	货号	规格
细胞活性检测 - 化学发光法	Cell Counting Kit - Luminescence	CK18	200T
细胞毒性检测	Cytotoxicity LDH Assay Kit - WST	CK12	500T
活死细胞双染	Calcein - AM/PI Double Staining Kit	C542	500T
细胞凋亡检测	Annexin V, FITC Apoptosis Detection Kit	AD10	50T
	Annexin V, 633 Apoptosis Detection Kit	AD11	50T
	Caspase - 3 Assay Kit - Colorimetric -	C551	50T
细胞周期检测	Cell Cycle Assay Kit - PI/RNase Staining	C543	50T
ROS检测	ROS Assay Kit - Highly Sensitive DCFH- DA	R252	100T
线粒体自噬检测	Mitophagy Detection Kit	MD01	1 set
线粒体染色	MitoBright LT Green, Red, Deep Red	MT10,11,12	20 $\mu$ l, 400 $\mu$ l
线粒体膜电位检测	JC - 1 MitoMP Detection Kit	MT09	1 set
线粒体超氧化物检测	MT - 1 MitoMP Detection Kit	MT13	1 set
	mtSOX Deep Red - Mitochondrial Superoxide Detection	MT14	1 set, 3 sets
免疫荧光用线粒体荧光染料	MitoBright IM Red for Immunostaining	MT15	20 $\mu$ l
脂质过氧化物检测	Liperfluo	L248	50 $\mu$ g, 50 $\mu$ g $\times$ 5
	MitoPeDPP	M466	5 $\mu$ g $\times$ 3
细胞二价铁离子(Fe <sup>2+</sup> )检测	FerroOrange	F374	1 tube, 3 tubes
	Mito - FerroGreen	M489	50 $\mu$ g $\times$ 2
组织内总铁含量检测	Iron Assay Kit - Colorimetric -	I291	50T
氧化型/还原型谷胱甘肽检测	GSSG/GSH Quantification Kit II	G263	100T

## Q&A:

### 1. 每孔应该接种多少细胞？

贴壁细胞每孔至少需要接种1,000个细胞 (100  $\mu$ l的培养基)，检测白细胞时由于它的灵敏度较低，每孔至少需要接种2,500个细胞 (100  $\mu$ l的培养基)，建议先做几个孔摸索接种细胞的数量。如果要使用24孔板或是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。

### 2. 如何设定空白对照？

在不含细胞的培养基中加入CCK-8，测定450 nm的吸光度即为空白对照。在做加药实验 (细胞毒性实验) 时，还应考虑药物的吸收，可在不含细胞，加入药物的培养基中加入CCK-8，测定450 nm的吸光度作为空白对照。

### 3. 哪些物质会影响CCK-8的测定？

当有还原性物质存在时会影响CCK-8的测定，增加O.D值；在有氧化性物质存在时会抑制测定反应的发生，减小O.D值；在有酚红存在的情况下，会增加空白吸收，但不影响测定，扣除空白吸收即可。

### 4. 在做加药实验时，药物对测定是否有影响？如何解决？

有时会有影响。如果药物具有还原性，就会和CCK-8试剂发生显色反应，增加吸光度。解决办法：首先要确认药物是否有吸收，在含有药物的培养基中加入CCK-8，测定450 nm的吸光度，如果它的吸光度比不含药物的培养基 (只加CCK-8) 的吸光度高，则证明药物有影响，可在加CCK-8之前更换培养基，去掉药物的影响。

### 5. CCK-8对细胞的毒性大小如何？

CCK-8对细胞的毒性相当低，同样的细胞在CCK-8法检测后还可用于其他细胞增殖的检测实验，如结晶紫检测法，中性红检测法或DNA荧光检测法等。

### 6. CCK-8的保质期有多久？

CCK-8在避光0-5  $^{\circ}$ C的条件下可以存放2年。在常温下可以保存3周左右，颜色应该为浅红色，如果颜色发生改变，则可能会增加空白吸光度。

### 7. 我没有450 nm的滤光片，还可以使用哪些其他的滤光片？

您可以使用吸光度在430-490 nm之间的滤光片，但是450 nm滤光片的检测灵敏度最高。

### 8. CCK-8能否对活细胞进行染色？

不能。因为CCK-8的主要成分是一种水溶性的四唑盐 (WST<sup>®</sup>-8)，并通过电子载体1-Methoxy PMS将活细胞中的电子交换到培养基中的WST<sup>®</sup>-8上，因为WST<sup>®</sup>-8及其生成的甲臞染料是高度水溶性的，不会进入细胞内，所以CCK-8不能对活细胞进行染色。

### 9. 必须设定参比波长吗？设定的目的是什么？

不一定要设定，CCK-8试剂在参比波长处没有吸光度。设定参比波长的目的是为了去除由于样品浑浊所带来的吸收。

### 10. 如果O.D值太低，可以采取什么办法？

可以采取2个办法：

适当增加细胞数量。

延长加入CCK-8试剂后的染色时间。

## CCK-8之Science篇:

1. X-ray screening identifies active site and allosteric inhibitors of SARS-CoV-2 main protease,  
*Science*, **2021**, 372, 642-646
2. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease,  
*Science*, **2020**, 368, 1331-1335
3. De novo design of potent and resilient hACE2 decoys to neutralize SARS-CoV-2,  
*Science*, **2020**, 370, 1208-1214



## CCK-8之Nature篇 (含子刊):

1. Chemical modification of PS-ASO therapeutics reduces cellular protein-binding and improves the therapeutic index, *Nature Biotechnology*, **2019**, 37640-650
2. Multi-omic measurements of heterogeneity in HeLa cells across laboratories, *Nature Biotechnology*, **2019**, 37, 314-322
3. A perfusable, multifunctional epicardial device improves cardiac function and tissue repair, *Nature Medicine*, **2021**, 27, 480-490
4. Dual effects of carbon monoxide on pericytes and neurogenesis in traumatic brain injury, *Nature Medicine*, **2016**, 22, 1335-1341
5. Human induced pluripotent stem cell-derived cardiomyocytes recapitulate the predilection of breast cancer patients to doxorubicin-induced cardiotoxicity, *Nature Medicine*, **2016**, 22(5), 547-556
6. Orphan nuclear receptor NR4A1 regulates transforming growth factor- $\beta$  signaling and fibrosis, *Nature Medicine*, **2015**, 21(2), 150-158
7. Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer, *Nature Medicine*, **2013**, 19, 329-336
8. Compromised CDK1 activity sensitizes BRCA-proficient cancers to PARP inhibition, *Nature Medicine*, **2011**, 17(7), 875-882
9. Mesenchymal stem cell-based tissue regeneration is governed by recipient T lymphocytes via IFN- $\gamma$  and TNF- $\alpha$ , *Nature Medicine*, **2011**, 17(12), 1594-1601
10. Monoclonal antibody targeting of N-cadherin inhibits prostate cancer growth, metastasis and castration resistance, *Nature Medicine*, **2010**, 16(12), 1414-1420
11. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche, *Nature Medicine*, **2007**, 13(10), 1219-1227
12. Altered TMPRSS2 usage by SARS-CoV-2 Omicron impacts infectivity and fusogenicity, *Nature*, **2022**, doi.org/10.1038/s41586-022-04474-x
13. DAXX represents a new type of protein-folding enabler, *Nature*, **2021**, 597, 132-137
14. DHODH-mediated ferroptosis defence is a targetable vulnerability in cancer, *Nature*, **2021**, 593, 586-590
15. Dietary fructose improves intestinal cell survival and nutrient absorption, *Nature*, **2021**, 597, 263-267
16. Epigenetic therapy inhibits metastases by disrupting premetastatic niches, *Nature*, **2020**, doi.org/10.1038/s41586-020-2054-x
17. ILC2s amplify PD-1 blockade by activating tissue-specific cancer immunity, *Nature*, **2020**, 579, 130-135
18. Potential circadian effects on translational failure for neuroprotection, *Nature*, **2020**, 582, 395-398
19. Chimeric peptidomimetic antibiotics against Gram-negative bacteria, *Nature*, **2019**, doi:10.1038/s41586-019-1665-6
20. PU.1 controls fibroblast polarization and tissue fibrosis, *Nature*, **2019**, 566, 344-349
21. Mitochondrial unfolded protein response controls matrix pre-RNA processing and translation, *Nature*, **2016**, 534, 710-713
22. Transfer of mitochondria from astrocytes to neurons after stroke, *Nature*, **2016**, 535, 551-555
23. Signalling thresholds and negative B-cell selection in acute lymphoblastic leukaemia, *Nature*, **2015**, 521, 357-361
24. The sonic hedgehog factor GLI1 imparts drug resistance through inducible glucuronidation, *Nature*, **2014**, 511(7507), 90-93
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2022年6月印刷