

## Enhanced Cell Counting Kit-8 (增强型CCK-8试剂盒)

| 产品编号  | 产品名称                                       | 包装   |
|-------|--|------|
| C0041 | Enhanced Cell Counting Kit-8 (增强型CCK-8试剂盒) | 100次 |

### 产品简介:

- Enhanced Cell Counting Kit-8, 简称增强型CCK-8试剂盒或增强型CCK8试剂盒, 是一种基于WST-8而广泛应用于细胞增殖和细胞毒性的快速、高灵敏度检测的试剂盒, 比普通的CCK-8试剂盒具有更好的检测灵敏度和更宽的线性范围。
- 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formazan (参考图1)。细胞增殖越多越快, 则颜色越深; 细胞毒性越大, 则颜色越浅。对于同样的细胞, 颜色的深浅和细胞数目呈线性关系。

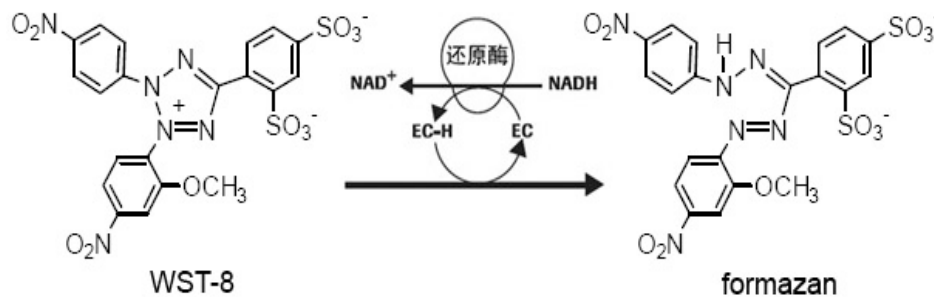


图1. WST-8检测原理图 (EC=electron coupling reagent, 即电子耦合试剂)

- WST-8是MTT的一种升级替代产品, 和MTT或其它MTT类似产品如XTT、MTS等相比有明显的优点。首先, MTT被线粒体内的一些脱氢酶还原生成的formazan不是水溶性的, 需要有特定的溶解液来溶解; 而WST-8和XTT、MTS产生的formazan都是水溶性的, 可以省去后续的溶解步骤。其次, WST-8产生的formazan比XTT和MTS产生的formazan更易溶解。再次, WST-8比XTT和MTS更加稳定, 使实验结果更加稳定。另外, WST-8和MTT、XTT等相比线性范围更宽, 灵敏度更高。
- WST-8和WST-1相比, 检测灵敏度更高, 更易溶解, 并且更加稳定。
- 本试剂盒可以用于细胞因子等诱导的细胞增殖检测, 也可以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。
- 本试剂盒检测非常便捷。试剂盒仅一管已经配制好的含有WST-8的增强型CCK-8溶液, 无须再进行任何配制等操作。无须使用同位素, 所有的检测步骤仅在同一块96孔板内完成。不必洗涤细胞, 不必收集细胞, 也不必采用额外的步骤去溶解formazan。可以用于大批量样品的检测。
- 酚红和血清对本试剂盒的测定无明显影响。
- WST-8对细胞无明显毒性。加入增强型CCK-8溶液显色后, 可以在不同时间反复用酶标仪读板, 使检测时间更加灵活, 便于找到最佳测定时间。
- 碧云天各种细胞增殖和细胞毒性检测试剂盒的比较和选择, 请参考<http://www.beyotime.com/support/cell-proliferation.htm>。
- 本试剂盒可以测定100个样品。

### 包装清单:

| 产品编号  | 产品名称       | 包装  |
|-------|------------|-----|
| C0041 | 增强型CCK-8溶液 | 1ml |
| —     | 说明书        | 1份  |

### 保存条件:

4°C避光保存一年有效, -20°C避光保存两年有效。

### 注意事项:

- 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。一方面, 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; 另一方面, 可以把96孔板置于靠近培养箱内水源的地方, 以缓解蒸发。
- 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。

- 用酶标仪检测前需确保每个孔内没有气泡，否则会干扰测定。
- 本产品仅限于专业人员的科学研究用，不得用于临床诊断或治疗，不得用于食品或药品，不得存放于普通住宅内。
- 为了您的安全和健康，请穿实验服并戴一次性手套操作。

### 使用说明：

1. 通常细胞增殖实验每孔加入100微升2000个细胞，细胞毒性实验每孔加入100微升5000个细胞(具体每孔所用的细胞的数目，需根据细胞的大小、细胞增殖速度的快慢等因素决定)。按照实验需要，进行培养并给予0-10微升特定的药物刺激。
2. 每孔加入10微升增强型CCK-8溶液。如果起始的培养体积为200微升，则需加入20微升增强型CCK-8溶液，其它情况以此类推。可以用加了相应量细胞培养液和增强型CCK-8溶液但没有加入细胞的孔作为空白对照。如果担心所使用的药物会干扰检测，需设置加了相应量细胞培养液、药物和增强型CCK-8溶液但没有加入细胞的孔作为空白对照。
3. 在细胞培养箱内继续孵育0.5-4小时，对于大多数情况孵育1小时就可以了。时间的长短根据细胞的类型和细胞的密度等实验情况而定，初次实验时可以在0.5、1、2和4小时后分别用酶标仪检测，然后选取吸光度范围比较适宜的一个时间点用于后续实验。
4. 在450nm测定吸光度。如无450nm滤光片，可以使用420-480nm的滤光片。可以使用大于600nm的波长，例如650nm，作为参考波长进行双波长测定。
5. 将不同数量的HeLa细胞按照每孔100微升培养液接种到96孔板中，培养至细胞贴壁充分后，每孔加入10微升的增强型CCK-8溶液孵育2小时后测定A450的检测效果图参考图2。检测效果仅供参考，实测数据会因检测仪器等的不同而存在差异。

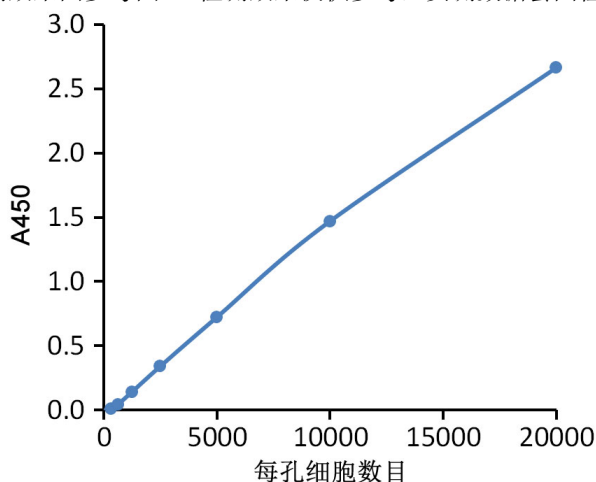


图2. 增强型CCK-8试剂盒测定不同数量HeLa细胞的检测效果图。实测数据会因检测仪器等的不同而存在差异，图中数据仅供参考。

### 常见问题：

#### 1. 增强型CCK-8溶液对细胞的毒性大小如何？

增强型CCK-8溶液对细胞的毒性非常低，和普通的CCK-8溶液相当或者毒性更低，通常观察不到细胞毒性。细胞在CCK-8法检测后仍然可以正常生长，并可以用于其它的细胞实验。但为了避免增强型CCK-8溶液可能带来的对于后续检测的影响，除非该细胞极难获得，否则不推荐把增强型CCK-8溶液孵育过的细胞用于其它实验。

#### 2. 如果吸光度值太低，可以采取什么办法？

- a. 适当增加细胞数量。
- b. 延长加入增强型CCK-8溶液后的孵育时间。

### 相关产品：

| 产品编号  | 产品名称                                       | 包装     |
|-------|--|--------|
| C0009 | MTT细胞增殖及细胞毒性检测试剂盒                          | 500次   |
| C0035 | WST-1细胞增殖及细胞毒性检测试剂盒                        | 100次   |
| C0036 | WST-1细胞增殖及细胞毒性检测试剂盒                        | 500次   |
| C0037 | Cell Counting Kit-8 (CCK-8试剂盒)             | 100次   |
| C0038 | Cell Counting Kit-8 (CCK-8试剂盒)             | 500次   |
| C0039 | Cell Counting Kit-8 (CCK-8试剂盒)             | 2500次  |
| C0040 | Cell Counting Kit-8 (CCK-8试剂盒)             | 10000次 |
| C0041 | Enhanced Cell Counting Kit-8 (增强型CCK-8试剂盒) | 100次   |
| C0042 | Enhanced Cell Counting Kit-8 (增强型CCK-8试剂盒) | 500次   |
| C0043 | Enhanced Cell Counting Kit-8 (增强型CCK-8试剂盒) | 2500次  |
| C0046 | Enhanced Cell Counting Kit-8 (增强型CCK-8试剂盒) | 10000次 |

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