V02

# 细胞增殖及细胞毒性检测试剂盒（中性红法）

**货号：**G1317
**规格：**500T/1000T
**保存：**2-8℃，避光保存，有效期 1 年。

## 产品组成：

| 名称 | 500T | 1000T | 保存 |
|---|---|---|---|
| 试剂(A)：中性红染色液 | 5mL | 10mL | 2-8℃，避光 |
| 试剂(B)：中性红裂解液 | 50mL | 100mL | 2-8℃ |

## 产品介绍：

细胞对中性红的摄入取决于活细胞细胞对 pH 梯度的维持能力。在生理 pH 条件下，中性红染料的净电荷几乎为零，从而使其能通过非离子被动扩散的方式穿透细胞膜进入细胞。溶酶体(lysosomes)中的质子梯度使溶酶体中的 pH 值低于细胞质，从而可以使中性红带上电荷并在溶酶体中积累。在细胞增殖加快时，细胞数量增多，可以摄入的中性红的量就会增加。在细胞受到损伤时，中性红的摄入能力会下降。经过一定时间摄入后，细胞经清洗并用裂解液裂解即可释放中性红而用于检测。这样通过测定细胞对于中性红的摄入量，就可以确定细胞的增殖或毒性情况。

细胞增殖及细胞毒性检测试剂盒（中性红法）是一种基于细胞对于中性红的摄入能力来检测细胞增殖或细胞毒性的试剂盒。本产品性质稳定，不易沉淀析出，可重复性良好。

## 自备材料：

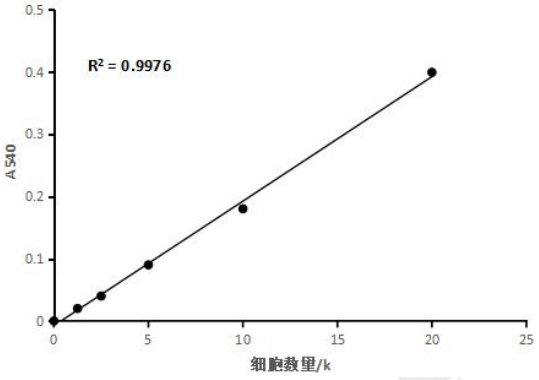
酶标仪或微量分光光度计、1×PBS

## 操作步骤：*（仅供参考）*

1. 把细胞培养在 96 孔培养板内，每孔加入 100ul 细胞培养液，设置适当的阴性对照和阳性对照，并给予药物刺激。（见注意事项 4）
2. 至待检测时间点，如果培养液中的药物推测不会对后续检测产生干扰，直接加入 10ul 中性红染色液；如果培养液中的药物可能会对后续检测产生干扰，先用 1×PBS 洗涤 1-2 次，随后加入 100ul 细胞培养液，并加入 10ul 中性红染色液，或直接将中性红染色液混入培养液后加入孔板。
3. 细胞培养箱内恒温孵育 2 小时。（见注意事项 2）
4. 去除含有中性红染色液的细胞培养液，用 1×PBS 洗涤 1-2 次。
5. 加入 100ul 中性红裂解液，室温裂解 10 分钟。在摇床上摇动可以促进样品的裂解萃取。
6. 在 540nm 测定裂解后液体吸光度值，可以选择 690nm 作为参考波长。

## 染色结果：

单种细胞的液泡系体积趋于一致，即在一定细胞数范围内，能够摄取的中性红的量与细胞数成线性相关。通过带入标曲可以对增殖和毒性损伤导致的细胞数量变化进行数字化统计。以 A549 细胞为例，检测效果如右：




## 注意事项：



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Address: No. 85, Liandong U Valley, Middle Zone, 101102, Tongzhou Dist, Beijing, China

1. 第一次使用本试剂盒时建议先取少量样品做好预实验。本产品实测数据会因细胞种类、细胞状态、检测仪器等的不同而存在差异。
2. 对于细胞密度非常低，细胞代谢速率非常慢的情况，可以把孵育时间延长到 3-4 个小时。
3. 中性红染色液长期存放可能会产生沉淀。可以通过离心吸取染色液的上清液使用，也可以使用针头滤器等过滤去除沉淀后继续使用，不会影响使用效果。因为染色液中的中性红本来就是过量的。
4. 96 孔板在细胞培养过程中会存在蒸发问题，可以在外围一圈每孔加入 100ul PBS 防止蒸发，以免对后续中性红吸收和数据检测造成干扰。
5. 为了您的安全和健康，请穿实验服并戴一次性手套操作。

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# Cell Counting Kit(Neutral Red Method)

**Cat:** G1317
**Size:** 500T/1000T
**Storage:** 2-8℃, avoid light, valid for 1 year.

## Kit Components

| Reagent | 500T | 1000T | Storage |
|---|---|---|---|
| Reagent(A)：CCK-N Stain Solution | 5mL | 10mL | 2-8℃, avoid light |
| Reagent(B)：CCK-N Extract Solution | 50mL | 100mL | 2-8℃ |

## Introduction

The uptake of neutral red by cells depends on the ability of living cells to maintain pH gradients. Under physiological pH conditions, the net charge of neutral red dye is almost zero, allowing it to penetrate the cell membrane and enter the cell through non ionic passive diffusion. The proton gradient in lysosomes causes the pH value in the lysosomes to be lower than the cytoplasm, which can charge neutral red and accumulate in the lysosomes. When cell proliferation accelerates, the number of cells increases, and the amount of neutral red that can be consumed will increase. When cells are damaged, the ability to absorb neutral red decreases. After a certain period of intake, the cells are washed and lysed with lysate to release neutral red for detection. By measuring the intake of neutral red by cells, the proliferation or toxicity of cells can be determined.

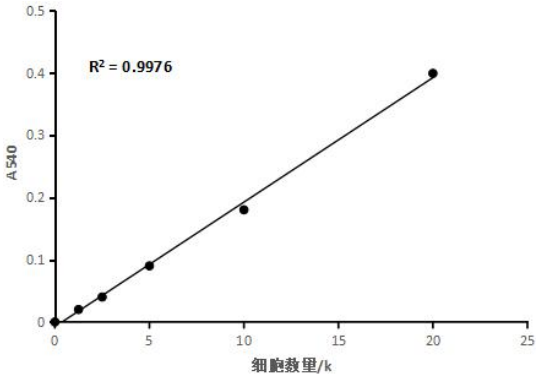
The Cell Counting Kit(Neutral Red Method) is a kit that detects cell proliferation or cytotoxicity based on the ability of cells to absorb neutral red. This product has stable properties, is not easy to precipitate, and has good repeatability.

## Protocol(*for reference only*)

1. Add 100μl of cell culture medium to each well of the 96 well plate, set appropriate negative and positive controls, and administer medication stimulation.(See Note 4)
2. At the time point to be tested, if it is speculated that the drugs in the culture medium will not interfere with subsequent testing, directly add 10ul of CCK-N Stain Solution; If the drugs in the culture medium may interfere with subsequent detection, first wash with 1×PBS 1-2 times, then add 100ul of cell culture medium and 10ul of CCK-N Stain Solution.Alternatively, CCK-N Stain Solution can be directly mixed into the culture medium and added to the well plate.
3. Incubate the cells in a constant temperature incubator for 2 hours. (See Note 2)
4. Remove the cell culture medium containing CCK-N Stain Solution and wash it 1-2 times with 1×PBS.
5. Add 100ul of CCK-N Extract Solution and crack at room temperature for 10 minutes. Shaking on a shaking table can promote the cracking and extraction of the sample.
6. When measuring the absorbance value of the liquid after cracking at 540nm, 690nm can be selected as the reference wavelength.

## Result

The volume of the vacuolar system of a single cell tends to be consistent, that is, within a certain range of cell numbers, the amount of neutral red that can be absorbed is linearly correlated with the number of cells. By bringing in the standard curve, digital statistics can be conducted on the changes in cell numbers caused by proliferation and toxic damage. Taking A549 cells as an example, the detection results are as follows:




## Note

1. When using this reagent kit for the first time, it is recommended to take a small amount of samples for



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preliminary experiments. The measured data of this product may vary due to differences in cell type, cell status, testing instruments, etc.

2. For situations where cell density is very low and cell metabolism rate is very slow, the incubation time can be extended to 3-4 hours.
3. Long term storage of CCK-N Stain Solution may result in precipitation. The supernatant of the staining solution can be extracted by centrifugation for use, or a needle filter can be used to filter and remove sediment before continuing use, without affecting the effectiveness of use. Because the neutral red in the staining solution is already excessive.
4. During the cell culture process, there may be evaporation issues with the 96 well plate. 100ul of PBS can be added to each well in the outer circle to prevent evaporation and avoid interference with subsequent neutral red absorption and data detection.
5. For your safety and health, please wear laboratory clothes and disposable gloves for operation.