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过氧化氢检测试剂盒

产品编号	产品名称	包装
S0038	过氧化氢检测试剂盒	150次

产品简介:

- 过氧化氢检测试剂盒(Hydrogen Peroxide Assay Kit)可以用于培养细胞或组织内过氧化氢水平的测定，也可以用于培养细胞的上清或血清、尿液、血浆或其它生物体液中的过氧化氢浓度的测定。
- 过氧化氢是一种活性氧代谢的副产物，在许多氧化应急反应中过氧化氢都是一种关键的调节因子。过氧化氢可以激活NF-κB等因子，这些过氧化氢相关的信号途径和哮喘、炎症性关节炎、动脉硬化以及神经退行性疾病等许多疾病相关。过氧化氢也和细胞凋亡、细胞增殖等密切相关。
- 本试剂盒通过过氧化氢氧化二价铁离子产生三价铁离子，然后和xylenol orange在特定的溶液中形成紫色的产物，从而实现对过氧化氢浓度的测定。本试剂盒经过改良配方，可以检测低达1微摩尔/升的过氧化氢。
- 本试剂盒方便快捷，通常10-20个样品可以在40-60分钟内测定完毕。
- 本试剂盒可以检测150个样品。

包装清单:

产品编号	产品名称	包装
S0038-1	过氧化氢检测试剂	15ml
S0038-2	过氧化氢标准溶液(1M)	1ml
S0038-3	过氧化氢检测裂解液	50ml
—	说明书	1份

保存条件:

-20°C保存，一年有效。其中过氧化氢标准溶液需避光保存。

注意事项:

- 一些干扰氧化还原的试剂或在酸性条件下呈紫色或接近的试剂会对过氧化氢的检测产生干扰，需尽量避免。
- 如果样品中含有外加的较高浓度的铁盐，会干扰测定。但普通培养基、血清等样品中含有的微量的铁盐不会干扰测定。
- 所测得的标准曲线尽管在一定的浓度范围内接近直线，但整个标准曲线不是直线。
- 测定时需可以测定A560的酶标仪一台(测540-570nm也可以)或可以测定微量样品的分光光度计一台。
- 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. 样品测定的准备:

a. 细胞或组织样品的制备

对于培养的细胞，先收集细胞到离心管内，弃上清，按照每100万细胞加入100-200微升过氧化氢检测裂解液的比例加入裂解液，随后充分匀浆以破碎并裂解细胞。4°C约12000g离心3-5分钟，取上清，用于后续测定。组织样品按照每5-10mg组织加入100-200微升裂解液的比例进行匀浆。4°C约12000g离心3-5分钟，取上清用于后续测定。以上所有操作均需在4°C或冰上操作。制备好的细胞或组织样品如果不立即测定，可以-20°C冻存。

b. 培养细胞上清液样品的制备

培养细胞的上清液可以直接用于后续的测定。

c. 血清、血浆或尿液样品的准备:

配制50mM磷酸缓冲液，pH为6.0。用pH为6.0的50mM磷酸缓冲液把样品稀释50倍。例如4微升样品稀释到196微升pH为6.0的50mM磷酸缓冲液中。稀释后即可用于后续的测定。

2. 标准曲线测定的准备:

a. 过氧化氢标准品的校准:

由于过氧化氢不是非常稳定，使用前需自行测定过氧化氢的实际浓度以进行校准。把浓度约为1M的过氧化氢用水稀释100倍，使过氧化氢的浓度约为10mM，测定A_{240}。A_{240}的测定可采用如下的任一方法:

(a) **普通紫外分光光度计法:** 使用含比色皿架的紫外分光光度计、NanoDrop 2000C、NanoDrop One^c、QuickDrop等仪器，

配套石英比色皿。确定比色皿光程(path length)，一般为1cm。用比色皿检测的过氧化氢浓度最接近实际浓度。

(b) **微量紫外分光光度计法：**如NanoDrop 2000、NanoDrop One、QuickDrop、含超微量检测板μDrop Plate的Varioskan等仪器。确定光程：对于NanoDrop 2000、NanoDrop One等，需要取消“自动化光程”，此时光程一般为0.1cm；Varioskan的超微量检测板μDrop Plate的光程一般为0.05cm。具体的微量紫外分光光度计的光程请参考仪器参数。

(c) **96孔紫外酶标仪法(须能检测240nm波长)：**根据96孔板的参数确定光程，一般200微升样品的光程为0.552cm (样品体积除以96孔单孔孔内横截面面积)。一般建议使用专用的96孔紫外检测板(如96孔UV板)，如果没有紫外检测板，也可使用一般的96孔板，但由于为非紫外检测专用板，会有非常高的紫外吸收信号，所以需要设置含等量双蒸水的孔作为空白对照(一般200μl水在该类96孔板的A_{240}在3.8左右)，计算时须减去该空白对照。在使用非紫外检测专用板的情况下，由于96孔酶标仪在240nm的检测上限有限，建议将过氧化氢稀释至约10mM左右后再进行浓度测定。

注意：以上所有方法都需要设置等量双蒸水作为空白对照，并在计算时减去该空白对照。

浓度计算公式：c=A/(ε×b)。其中：c为样品浓度(单位为mol/L或M)；A为吸光值；ε为波长依赖的摩尔消光系数(单位为$L\times mol^{-1}\times cm^{-1}$或$M^{-1}\times cm^{-1}$)，过氧化氢的摩尔消光系数为$43.6M^{-1}cm^{-1}$；b=光程(单位为cm)。

因此：过氧化氢浓度(M)=A_{240}/(43.6×b)；即：过氧化氢浓度(mM)=22.94×A_{240}/b

从而计算出本试剂盒提供的过氧化氢的实际浓度，并根据实际测定出来的浓度进行后续的标准曲线的设置。

示例：将本试剂盒提供的约为1M的过氧化氢用双蒸水稀释100倍后，用96孔酶标仪及一般的96孔板进行检测，每孔200微升，每组3个平行。双蒸水对照组的平均A_{240}为3.750，过氧化氢样品组的平均A_{240}为3.974，差值为0.224，200微升样品的光程为0.552cm。代入公式，过氧化氢浓度(mM)=22.94×0.224/0.552=9.31，则实际本试剂盒提供的过氧化氢浓度为0.931M。

b．标准曲线的设置：

样品在什么溶液中标准品也需用什么溶液稀释，这样可以减小误差。例如对于细胞样品，标准品宜用过氧化氢检测裂解液稀释，对于培养细胞的上清液样品，标准品宜用相应的细胞培养液稀释。标准溶液可以稀释成1、3、10、30、100微摩尔/升，或1、2、5、10、20、50、100微摩尔/升，初次测定后知道样品的浓度范围后可以对标准品在样品浓度范围附近密集测定。

3．过氧化氢浓度的测定：

a．把过氧化氢检测试剂在冰上或冰水浴上融解。

b．在检测孔或检测管内加入50微升样品或标准品。

c．在每个孔内加入100微升过氧化氢检测试剂。

d．轻轻振荡或敲打混匀，室温(15-30°C)放置30分钟。然后立即测定A560。如测A560有困难，波长可以选择540- 570nm。

e．根据标准曲线计算出样品中过氧化氢的浓度。

注意：如果样品中过氧化氢的浓度过高，可以适当稀释后再测定。如果样品中过氧化氢的浓度过低，可以把样品的体积改为使用100微升，同时标准品也使用100微升，而检测试剂仍然使用100微升。这样可以提高检测的灵敏度，但缺点是样品需要消耗100微升。

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