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碧云天生物技术/Beyotime Biotechnology
订货热线: 400-1683301 或 800-8283301
订货 e-mail: order@beyotime.com
技术咨询: info@beyotime.com
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超氧化物检测试剂盒

产品编号	产品名称	包装
S0060	超氧化物检测试剂盒	100次

产品简介:

- 超氧化物检测试剂盒(Superoxide Assay Kit)是一种用于超氧化物快速高灵敏度检测的试剂盒。
- 本试剂盒利用超氧化物可以还原WST-1产生可溶性有色物质为基础来检测超氧化物。本试剂盒的检测试剂中添加了Catalase(触酶)等，可以清除过氧化氢等过氧化物对WST-1显色的干扰，使测定结果更加准确。并且本试剂盒还提供SOD(超氧化物歧化酶)，可以验证本试剂盒测定出的结果是否为超氧化物，以排除检测体系中所用的一些试剂可能产生的干扰。对于本试剂盒，加入SOD后通常可以抑制90%以上的超氧化物。
- WST-1是一种类似于MTT的化合物，可以被超氧化物还原生成橙色的formazan。超氧化物产生越多越快，则颜色越深，即相应测定得到的吸光度值越大。
- 使用WST-1作为检测试剂比用传统的细胞色素c (cytochrome c)检测超氧化物有多方面的优点。首先，WST-1本身的吸光度非常低(通常OD450为0.02左右，因仪器和酶标板不同而有所不同)，而细胞色素c本身的吸光度非常高(通常OD550为0.5左右)，因此，使用WST-1与细胞色素c相比，检测灵敏度高很多倍。其次，WST-1的还原产物是稳定的可溶性产物，且对细胞基本无毒性，使WST-1方法更加适合于高通量的筛选。另外，细胞色素c法由于灵敏度的限制，一般不适合用96孔板测定，而WST-1法可以用96孔板测定，使检测更加便捷。用WST-1法或细胞色素c法对超氧化物测定效果比较参考图1。图1中，50万/毫升嗜中性粒细胞在37°C用PMA刺激指定时间，用WST-1或细胞色素c方法分别测定。WST-1法测定OD450，而细胞色素c法测定OD550。另外，WST-1法和luminol法相比，灵敏度相近，但仅须使用普通的酶标仪，无须使用luminol法所需的luminometer，并且检测速度也比luminol法更加快捷。
- 超氧化物通常指超氧化物阴离子(superoxide anion) $O_2^{\cdot-}$，是一种氧分子的自由基。在呼吸链中，NADPH氧化酶把电子传递给氧分子的时候，就会产生超氧化物阴离子$O_2^{\cdot-}$。超氧化物阴离子$O_2^{\cdot-}$是一种强氧化剂，可以由被刺激的白细胞等产生，从而抵御微生物的感染等。超氧化物阴离子$O_2^{\cdot-}$也可以导致氧化损伤，和许多疾病的发生密切相关。
- 本试剂盒可以测定100个样品。

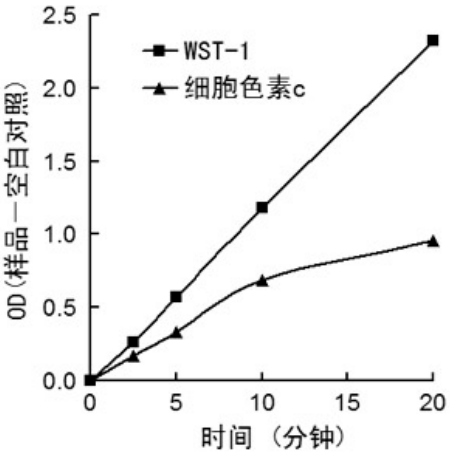

图 1. WST-1 法和细胞色素 c 法对于超氧化物测定效果的比较。

包装清单:

产品编号	产品名称	包装
S0060-1	超氧化物检测缓冲液	30ml
S0060-2	WST-1溶液	1ml
S0060-3	Catalase溶液	200μl
S0060-4	SOD溶液	20μl
—	说明书	1份

保存条件:

-20°C保存，一年有效。

注意事项:

- WST-1溶液、Catalase溶液和SOD溶液均宜尽量避免反复冻融，可以适当分装。
- 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. 超氧化物检测工作液的配制:
   按照下表比例配制超氧化物检测工作液:

	1个检测	5个检测	10个检测	20个检测	50个检测

超氧化物检测缓冲液	200微升	1毫升	2毫升	4毫升	10毫升
WST-1溶液	10微升	50微升	100微升	200微升	500微升
Catalase溶液	2微升	10微升	20微升	40微升	100微升
超氧化物检测工作液	212微升	1.06毫升	2.12毫升	4.24毫升	10.6毫升

2. 悬浮细胞产生超氧化物的检测：
   a. 细胞用PBS、Hanks液或生理盐水洗涤一次。
   b. 约按5-10万个细胞/毫升的比例，用超氧化物检测工作液悬浮细胞。
   c. 在96孔板中，每孔加入200微升用超氧化物检测工作液悬浮的细胞，每孔约1-2万个细胞。
   d. 37°C孵育3分钟。
   e. 在预定的检测孔中加入预计可以诱导产生超氧化物的刺激物，如PMA等，刺激10-60分钟或其它预定的时间。须至少留一个不加刺激物的孔作为空白对照。选择1-2个加刺激物的孔中加入2微升SOD以验证整个检测体系，加SOD溶液的检测可以不做。试剂盒提供的SOD溶液足够做10个检测。具体检测时的安排参考下表：

	细胞	超氧化物检测工作液	刺激物	SOD溶液
空白对照	+	+	−	−
待测样品	+	+	+	−
阴性对照	+	+	+	+

   f. 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的波长作为参考波长进行双波长测定。
3. 贴壁细胞产生超氧化物的检测：
   a. 约按每孔5000-10000个细胞(具体的细胞接种数量须根据细胞的大小和生长速度自行确定)把细胞培养到96孔板中，使第二天或待检测时细胞为80-90%满。
   b. 吸去培养液，用PBS、Hanks液或生理盐水洗涤一次。
   c. 每孔加入200微升超氧化物检测工作液，37°C孵育3分钟。
   d. 在预定的检测孔中加入预计可以诱导产生超氧化物的刺激物，如PMA等，刺激10-60分钟或其它预定的时间。须至少留一个不加刺激物的孔作为空白对照。选择1-2个加刺激物的孔中加入2微升SOD以验证整个检测体系，加SOD溶液的检测可以不做。试剂盒提供的SOD溶液足够做10个检测。具体检测时的安排参考下表：

	细胞	超氧化物检测工作液	刺激物	SOD溶液
空白对照	+	+	−	−
待测样品	+	+	+	−
阴性对照	+	+	+	+

   e. 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的波长作为参考波长进行双波长测定。

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