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活性氧检测试剂盒

产品编号	产品名称	包装
S0033	活性氧检测试剂盒	>100次

产品简介:

- 活性氧检测试剂盒(Reactive Oxygen Species Assay Kit, 也称ROS Assay Kit)是一种利用荧光探针DCFH-DA进行活性氧检测的试剂盒。DCFH-DA本身没有荧光,可以自由穿过细胞膜,进入细胞内后,可以被细胞内的酯酶水解生成DCFH。而DCFH不能通透细胞膜,从而使探针很容易被装载到细胞内。细胞内的活性氧可以氧化无荧光的DCFH生成有荧光的DCF。检测DCF的荧光就可以知道细胞内活性氧的水平。
- 本试剂盒提供了活性氧阳性对照试剂Rosup,以便于活性氧的检测。Rosup是一种混合物(compound mixture),浓度为50mg/ml。
- 本试剂盒本底低,灵敏度高,线性范围宽,使用方便。
- 本试剂盒可以测定100-500个样品。

包装清单:

产品编号	产品名称	包装
S0033-1	DCFH-DA (10mM)	0.1ml
S0033-2	活性氧阳性对照(Rosup, 50mg/ml)	1ml
—	说明书	1份

保存条件:

-20°C保存,一年有效。

注意事项:

- 探针装载后,一定要洗净残余的未进入细胞内的探针,否则会导致背景较高。
- 探针装载完毕并洗净残余探针后,可以进行激发波长的扫描和发射波长的扫描,以确认探针的装载情况是否良好。DCF的激发光谱和发射光谱请参考下图。
- 尽量缩短探针装载后到测定所用的时间(刺激时间除外),以减少各种可能的误差。
- 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. 装载探针

对于刺激时间较短(通常为2小时以内)的细胞,先装载探针,后用活性氧阳性对照或自己感兴趣的药物刺激细胞。对于细胞刺激时间较长(通常为6小时以上)的细胞,先用活性氧阳性对照或自己感兴趣的药物刺激细胞,后装载探针。

原位装载探针: 本方法仅适用于贴壁培养细胞。按照1:1000用无血清培养液稀释DCFH-DA,使终浓度为10微摩尔/升。去除细胞培养液,加入适当体积稀释好的DCFH-DA。加入的体积以能充分盖住细胞为宜,通常对于六孔板的一个孔加入稀释好的DCFH-DA不少于1毫升。37°C细胞培养箱内孵育20分钟。用无血清细胞培养液洗涤细胞三次,以充分去除未进入细胞内的DCFH-DA。通常活性氧阳性对照在刺激细胞20-30分钟后可以显著提高活性氧水平。

收集细胞后装载探针: 按照1:1000用无血清培养液稀释DCFH-DA,使终浓度为10微摩尔/升。细胞收集后悬浮于稀释好的DCFH-DA中,细胞浓度为二百万至二千万/毫升,37°C细胞培养箱内孵育20分钟。每隔3-5分钟颠倒混匀一下,使探针和细胞充分接触。用无血清细胞培养液洗涤细胞三次,以充分去除未进入细胞内的DCFH-DA。直接用活性氧阳性对照或自己感兴趣的药物刺激细胞,或把细胞等分成若干份后刺激细胞。通常活性氧阳性对照在刺激细胞20-30分钟后可以显著提高活性氧水平。

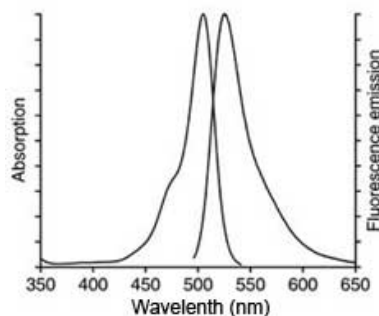
说明: 仅在阳性对照孔中加入Rosup作为阳性对照,其余孔不必加入Rosup。

2. 检测

对于原位装载探针的样品可以用激光共聚焦显微镜直接观察,或收集细胞后用荧光分光光度计、荧光酶标仪或流式细胞仪检测。对于收集细胞后装载探针的样品可以用荧光分光光度计、荧光酶标仪或流式细胞仪检测,用激光共聚焦显微镜直接观察也可以。

3. 参数设置

使用488nm激发波长,525nm发射波长,实时或逐时间点检测刺激前后荧光的强弱。DCF的荧光光谱和FITC非常相似,可以用FITC的参数设置检测DCF。DCF的激发光谱和发射光谱参考下图。



4. 其它说明

阳性对照可以按照1:1000的比例使用。例如装载好探针的细胞共1毫升，可以加入1微升的阳性对照刺激。通常刺激后20-30分钟内可以观察到非常显著的活性氧水平升高。对于不同的细胞，活性氧阳性对照的效果可能有较大的差别。如果在刺激后30分钟内观察不到活性氧的升高，可以适当提高活性氧阳性对照的浓度。如果活性氧升高得过快，可以适当降低活性氧阳性对照的浓度。

另外，对于某些细胞，如果发现没有刺激的阴性对照细胞荧光也比较强，可以按照1:2000-1:5000稀释DCFH-DA，使装载探针时DCFH-DA的浓度为2-5微摩尔/升。

探针装载的时间也可以根据情况在15-60分钟内适当进行调整。

活性氧阳性对照(Rosup)仅仅用于作为阳性对照的样品，并不是在每个样品中都需加入活性氧阳性对照。

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Version 2017.12.12