

## 活性氧检测试剂盒

产品编号	产品名称	包装
S0033S	活性氧检测试剂盒	>100次
S0033M	活性氧检测试剂盒	>500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[1, 2]。
- 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。使用本试剂盒检测细胞内活性氧的效果请参考图1。

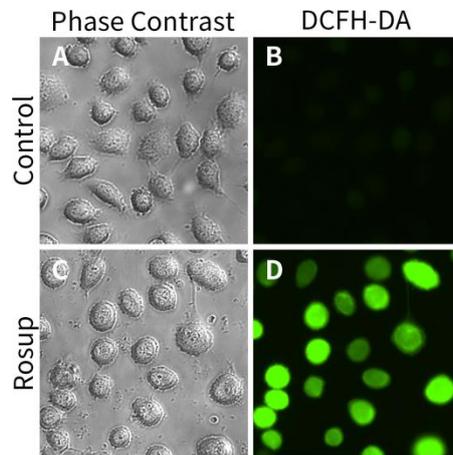


图1. 碧云天活性氧检测试剂盒(S0033)检测L929 (小鼠成纤维细胞)细胞内活性氧的效果图。L929细胞装载DCFH-DA探针后用50  $\mu\text{g/ml}$  Rosup处理30分钟,随后PBS洗涤一次后用荧光显微镜检测。正常的L929细胞中探针几乎未被氧化成荧光化合物,细胞中的绿色荧光非常弱(图A, B);使用活性氧阳性对照试剂Rosup处理使细胞内活性氧生成大幅增加, DCFH-DA与活性氧反应,细胞中的绿色荧光显著增强(图C, D)。实际结果会因实验条件、检测仪器等的不同而存在差异,图中效果仅供参考。

- 本试剂盒本底低,灵敏度高,线性范围宽,使用方便。
- 碧云天同时提供含优化的DCFH-DA探针稀释液和Rosup稀释液的活性氧检测试剂盒(含稀释液)(S0034),使用更便捷,通常可以确保获得更稳定可靠的检测效果。
- 本试剂盒小包装和中包装,6孔板每孔检测体系的体积为1ml时,分别可以检测100次和500次;96孔板每孔检测体系为100 $\mu\text{l}$ 时,分别可以检测1000次和5000次。如果用于流式细胞仪,每个样品检测体系体积为0.5ml时,分别可以检测200次和1000次。

### 包装清单:

产品编号	产品名称	包装
S0033S-1	DCFH-DA (10mM)	0.1ml
S0033S-2	活性氧阳性对照(Rosup, 50mg/ml)	0.1ml
—	说明书	1份

产品编号	产品名称	包装
S0033M-1	DCFH-DA (10mM)	0.5ml
S0033M-2	活性氧阳性对照(Rosup, 50mg/ml)	0.5ml
—	说明书	1份

### 保存条件:

-20 $^{\circ}\text{C}$ 保存,一年有效。其中DCFH-DA (10mM)须避光保存。

## 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页图谱。
- 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。
- 荧光酶标仪检测时须使用适合荧光检测的黑板或白板,推荐使用碧云天BeyoGold™全黑96孔细胞培养板(FCP966)或BeyoGold™黑色透明底96孔细胞培养板(平底带盖,独立包装)(FCP965)。
- 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用细胞外液(C0216)或酌情使用PBS、HBSS等适当溶液稀释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。用细胞外液(C0216)或酌情使用PBS、HBSS、无血清细胞培养液等适当溶液洗涤细胞三次,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用细胞外液(C0216)或酌情使用PBS、HBSS等适当溶液稀释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。用细胞外液(C0216)或酌情使用PBS、HBSS、无血清细胞培养液等适当溶液洗涤细胞三次,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。细胞外液推荐使用细胞外液(Extracellular Solution)(C0216),PBS推荐使用PBS(C0221A),HBSS推荐使用Hanks' Balanced Salt Solution(C0218)。碧云天同时提供含经优化的DCFH-DA探针稀释液和Rosup稀释液的活性氧检测试剂盒(含稀释液)(S0034),使用更便捷,通常可以确保获得更稳定可靠的检测效果。

### 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。

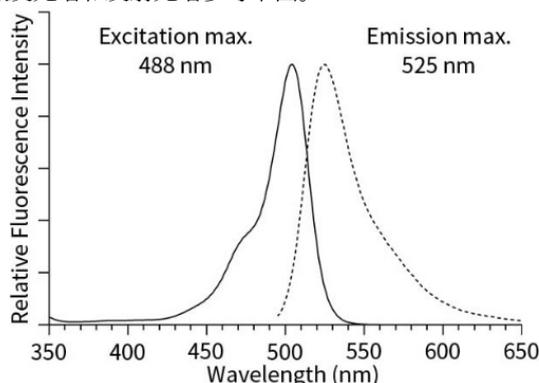


图2. DCF的激发光谱和发射光谱。

### 4. 其它说明

阳性对照Rosup可以按照1:1000的比例酌情使用PBS、HBSS、细胞外液或无血清培养液稀释后使用。例如装载好探针的细胞,直接吸除液体或离心后吸除液体,加入适当体积的按照1:1000稀释的Rosup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。Rosup对不同细胞的效果会有所不同。碧云天同时提供含经优化的Rosup稀释液的活性氧检测试剂盒(含稀释液)(S0034),使用更便捷,通常可以确保获得更稳定可靠的检测效果。

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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### 相关产品:

产品编号	产品名称	包装
C2055	BODIPY 500/510 C1, C12(脂肪酸绿色荧光探针)	1mg/5mg/25mg
S0019	DAF-FM DA (NO荧光探针)	>100次/>500次
S0033	活性氧检测试剂盒	>100次/>500次
S0034	活性氧检测试剂盒(含稀释液)	>100次/>500次
S0035	活性氧检测试剂盒(CM-H <sub>2</sub> DCFDA)	20-200次/100-1000次
S0036	活性氧检测试剂盒(CM-H <sub>2</sub> DCFDA, 含稀释液)	20-200次/100-1000次
S0038	过氧化氢检测试剂盒	150次
S0043	脂质过氧化检测试剂盒(BODIPY 581/591 C11)	100-1000次/500-5000次
S0051	过氧化氢酶检测试剂盒	100次
S0060	超氧化物检测试剂盒	100次
S0061	线粒体超氧化物检测试剂盒(MitoSOX Red)	20-200次/100-1000次
S0063	Dihydroethidium (超氧化物阴离子荧光探针)	5mg
S0064S	超氧阴离子活性氧检测试剂盒(DHE)	100-1000次
S0067-100μg	SOSG (单线态氧绿色荧光探针)	100μg
S0068S	单线态氧检测试剂盒(SOSG)	30-300次
S0131	脂质氧化(MDA)检测试剂盒	100/500次

### 使用本产品的文献:

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