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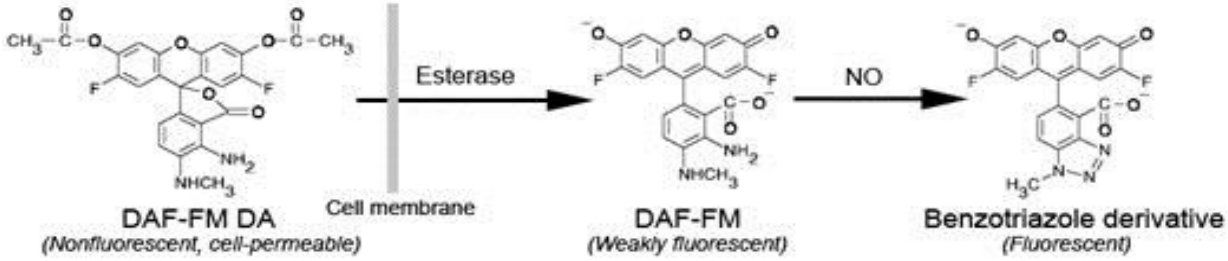
DAF-FM DA (NO荧光探针)

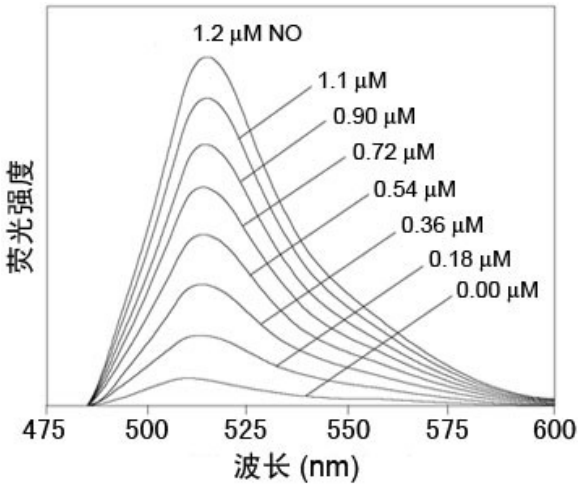
产品编号	产品名称	包装
S0019S	DAF-FM DA (NO荧光探针)	>100次
S0019M	DAF-FM DA (NO荧光探针)	>500次

产品简介：

- DAF-FM DA即3-Amino,4-aminomethyl-2',7'-difluorescein, diacetate，也称DAF-FM diacetate或4-Amino, 5-aminomethyl-2', 7'-difluorescein, diacetate。DAF-FM DA是最新一代用于一氧化氮定量检测的荧光探针，比以前比较常用的一氧化氮荧光检测探针DAF-2 diacetate有多方面的改进。首先，DAF-FM DA和DAF-2 diacetate相比，最后和一氧化氮反应形成的荧光产物受pH值的影响小，在pH值大于5.5时不受pH值的影响。其次，DAF-FM DA和DAF-2 diacetate相比，前者产生的荧光更加稳定，不容易淬灭，这样更加便于检测。另外，DAF-FM DA和DAF-2 diacetate相比，前者对一氧化氮的检测灵敏度更高，相同条件下检测灵敏度可以提高接近2倍，最低检测浓度可以达到3nM。



- DAF-FM DA可以穿过细胞膜(cell-permeable)，进入细胞后可以被细胞内的酯酶催化形成不能穿过细胞膜的DAF-FM 。DAF-FM本身仅有很弱的荧光，但在和一氧化氮反应后可以产生强烈荧光，激发波长为495nm，发射波长为515nm。DAF-FM DA检测一氧化氮的机制可以参考上图。任何可以检测fluorescein的仪器，包括荧光显微镜、激光共聚焦显微镜、流式细胞仪、荧光分光光度计或荧光酶标仪都可以用于该荧光探针的检测。右图为DAF-FM在不同浓度一氧化氮存在时的发射荧光扫描图谱(fluorescence emission spectra)。
- DAF-FM DA的分子量为496.4，分子式为$C_{25}H_{18}F_2N_2O_7$，HPLC分析纯度大于98%。
- 本DAF-FM DA为溶解于DMSO的淡黄色溶液，浓度为5mM。
- 本荧光探针适合于检测细胞内的一氧化氮水平，可以进行实时检测。如果收集细胞后再装载探针，通常S包装至少可以检测100个样品，M包装至少可以检测500个样品。



包装清单：

产品编号	产品名称	包装
S0019S-1	DAF-FM DA (NO荧光探针) 5mM	20μl
S0019S-2	DAF-FM DA稀释液	50ml
—	说明书	1份

产品编号	产品名称	包装
S0019M-1	DAF-FM DA (NO荧光探针) 5mM	100μl
S0019M-2	DAF-FM DA稀释液	250ml
—	说明书	1份

保存条件：

-20ºC保存，DAF-FM DA需避光保存，一年有效。

注意事项：

- BSA和酚红(phenol red)对本荧光探针的检测有干扰，需避免。
- 第一次使用时请分装成小包装后-20ºC保存，以避免反复冻融。
- 荧光探针应随用随配。配制好的探针工作液应立即使用，不能以后再用。
- DAF-FM DA在4ºC、冰浴等较低温度情况下会凝固而粘在离心管管底、管壁或管盖内，可以20-25ºC水浴温育片刻至全部融解后使用。
- 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，后用适当的阳性对照及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，先用适当的阳性对照及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比例，用本试剂盒提供的DAF-FM DA稀释液稀释DAF-FM DA，使终浓度为5微摩尔/升。去除细胞培养液，加入适当体积稀释好的DAF-FM 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DAF-FM DA的体积为1毫升。37ºC细胞培养箱内孵育20分钟。用PBS(pH7.4)洗涤细胞三次，以充分去除未进入细胞内的DAF-FM DA。

收集细胞后装载探针：按照1:1000比例，用本试剂盒提供的DAF-FM DA稀释液稀释DAF-FM DA，使终浓度为5微摩尔/升。细胞收集后，用稀释好的DAF-FM DA重悬细胞，细胞浓度为一百万至二千万/毫升，37ºC细胞培养箱内孵育20分钟。上述操作可以在离心管内进行。每隔3-5分钟颠倒混匀一下，使探针和细胞充分接触。用PBS(pH7.4)洗涤细胞三次，以充分去除未进入细胞内的DAF-FM DA。直接用适当的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再刺激细胞。

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(用普通的荧光显微镜观察效果相对较差)，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495nm激发波长，515nm发射波长，实时、逐时间点或单时间点检测刺激前后荧光的强弱。DAF-FM和一氧化氮反应产物的荧光光谱和fluorescein非常相似，可以用检测fluorescein的参数设置进行检测，用检测FITC的参数设置进行检测也可以。

4. 其它说明

上述推荐的DAF-FM DA的工作浓度为5微摩尔/升，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的比例稀释DAF-FM DA，使装载探针时DAF-FM DA的浓度为1-2.5微摩尔/升。相反，如果发现用感兴趣的药物刺激后荧光较弱，可以把DAF-FM DA的工作浓度为调整为10微摩尔/升，以提高检测的灵敏度。另外，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。

相关产品：

产品编号	产品名称	包装
S0006	L-NAME (eNOS抑制剂)	200mg
S0007	L-Canavanine (iNOS抑制剂)	20mg
S0008-100mg	SMT (iNOS抑制剂)	100mg
S0008-1g	SMT (iNOS抑制剂)	1g
S0009	1400W (iNOS抑制剂)	2mg
S0010	Spermidine (nNOS抑制剂)	200mg
S0011	L-NMMA (总NOS抑制剂)	5mg
S0012	L-Arginine (NO前体)	5g
S0015	SNP (NO供体)	1g
S0016	L-Citrulline (NO中间体)	1g
S0017	Hemoglobin (NO清除剂)	1g
S0019	DAF-FM DA (NO荧光探针)	>100次
S0021S	一氧化氮检测试剂盒	500次
S0021M	一氧化氮检测试剂盒	2500次
S0023	总一氧化氮检测试剂盒	50次
S0024	总一氧化氮检测试剂盒	200次
S0025	一氧化氮合成酶检测试剂盒(荧光法)	100次
S1546	Carboxy-PTIO (一氧化氮清除剂)	5mg
S1547	Carboxy-PTIO (一氧化氮清除剂)	50mg
S3090	细胞与组织裂解液(一氧化氮检测用)	100ml

ST1555-500mg	L-NAME (≥98%, Reagent grade)	500mg
ST1555-2g	L-NAME (≥98%, Reagent grade)	2g
ST1555-10g	L-NAME (≥98%, Reagent grade)	10g

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