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总一氧化氮检测试剂盒

产品编号	产品名称	包装
S0023	总一氧化氮检测试剂盒	50次

产品简介：

- 总一氧化氮检测试剂盒(Total Nitric Oxide Assay Kit，即Nitrate/Nitrite Assay Kit)采用了硝酸盐还原酶(Nitrate reductase)还原硝酸盐(nitrate)为亚硝酸盐(nitrite)，然后通过经典的Griess reagent检测亚硝酸盐，从而测定出总一氧化氮。一氧化氮本身极不稳定，在细胞内很快代谢为硝酸盐(Nitrate)和亚硝酸盐(Nitrite)，通过上述方法检测出硝酸盐和亚硝酸盐的总量，就可以推算出总的一氧化氮的量。
- 本试剂盒采用了NADPH依赖性硝酸盐还原酶(NADPH dependent nitrate reductase)。高浓度的NADPH会干扰后续的检测，消除NADPH的一种常用方法就是使用Lactate dehydrogenase (LDH)清除NADPH。本试剂盒采用了LDH清除NADPH的方法，使检测结果更加准确。
- 对亚硝酸盐的检测下限达到2微摩尔/升，在2-80微摩尔/升的范围内有很好的线性关系。浓度过高的样品可以适当稀释后再进行检测。
- 样品范围广，可以检测细胞裂解液、组织裂解液、细胞或组织的培养液、血清、血浆或尿液等中一氧化氮的含量。酚红和10%血清对测定无明显干扰。
- 样品需要量少。根据样品中一氧化氮的浓度不同，仅需0-60微升样品。
- 检测速度快，仅需约80分钟即可完成检测。
- 本试剂盒采用了间接的一氧化氮检测方法，如需检测细胞内实际的一氧化氮水平，可以采用碧云天生产的DAF-FM DA (NO荧光探针)(S0019)。

包装清单：

产品编号	产品名称	包装
S0023-1	样品稀释液	15ml
S0023-2	NADPH	5mg
S0023-3	FAD	550μl
S0023-4	Nitrate Reductase	250μl
S0023-5	LDH Buffer	550μl
S0023-6	LDH	500μl
S0023-7	$NaNO_2$ (1M)	1ml
S0023-8	Griess Reagent I	3ml
S0023-9	Griess Reagent II	3ml
—	说明书	1份

保存条件：

-20°C保存，一年有效。NADPH, Nitrate Reductase, $NaNO_2$ (1M), Griess Reagent I 和Griess Reagent II需避光保存。NADPH配制成溶液后必须分装并-70°C保存。

注意事项：

- RPMI 1640等含有较高浓度硝酸盐的培养液容易对本试剂盒的检测产生干扰，请尽量避免。必需使用RPMI 1640培养液时，在检测一氧化氮前必需先换成其他适当培养液例如DMEM、MEM、F12等，或换成HBSS或PBS等。
- 推荐选购碧云天生产的细胞与组织裂解液(一氧化氮检测用)(S3090)用于细胞或组织样品的裂解。也可以自行选择适当方式制备细胞或组织样品的裂解液用于总一氧化氮的检测，但需注意自行选择的方式可能会干扰试剂盒检测时的酶反应而影响检测效果。检测细胞或组织的培养液、血清、血浆或尿液中的总一氧化氮含量时，不需要选购S3090。S3090裂解获得的样品还可以用于蛋白浓度的检测以及Western印迹检测，但需注意S3090中不含蛋白酶和磷酸酯酶抑制剂。
- 检测反应必须避光进行。
- 由于检测过程中有还原反应，凡是影响还原反应的氧化或还原试剂要注意避免，例如常用的还原剂DTT和巯基乙醇。
- 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. 样品处理：

含有高浓度蛋白的样品例如血清、含有高浓度血清的细胞培养液，在加入Griess Reagent I后可能会产生沉淀。可以取50微升样品，加入50微升Griess Reagent I进行测试，观察是否产生沉淀。如果产生沉淀，则可以把样品沸水浴加热5分钟，以变性蛋白，然后12,000g离心5分钟，取上清用于后续的测定。对于细胞或组织样品可以用碧云天生产的细胞与组织裂解液(一氧化氮检测用)(S3090)进行裂解。对于尿液样品，通常需要用水稀释10-50倍后检测。不宜使用肝素抗凝的血浆，肝素抗凝的血浆在和Griess Reagent反应时会产生沉淀。

2. 稀释标准品：

用制备或稀释样品时所使用的溶液把1M $NaNO_2$稀释成2、5、10、20、40、60、80微摩尔/升。例如样品为使用S3090裂解获得的细胞裂解液，则宜使用S3090稀释标准品；样品为细胞培养液，则用该培养液稀释标准品。如果样品为血清，则可以使用本试剂盒提供的样品稀释液(S0023-1)或简单地使用PBS、生理盐水等适当溶液稀释标准品。稀释的标准品宜现配现用，不宜冻存后使用。

3. 试剂的准备：

a. 加约1ml双蒸水或Milli-Q级纯水至5mg NADPH中，颠倒混匀溶解后，再用双蒸水或Milli-Q级纯水定容至3ml，配制成2mM NADPH，除立即使用的部分外，其余NADPH溶液必须立即分装后-70°C冻存。

b. FAD已经配制在适当溶液中。FAD可以适当分装后-20°C或-70°C保存。

c. Nitrate Reductase和LDH在临用前取出，并放置在冰浴上使用(注意在使用完毕后立即-20°C保存)，试剂盒中的其余各种试剂在溶解后保存在冰浴上。Griess Reagent I和Griess Reagent II在使用前需达到室温。

4. 参考下表依次加入标准品、样品和检测试剂并进行相应检测：

	空白对照	标准品	样品
标准品	—	60微升	—
样品	—	—	x微升
用于样品稀释的溶液	60微升	—	(60-x) 微升
NADPH (2mM)	5微升	5微升	5微升
FAD	10微升	10微升	10微升
Nitrate Reductase	5微升	5微升	5微升
混匀后，37°C孵育30分钟			
LDH Buffer	10微升	10微升	10微升
LDH	10微升	10微升	10微升
混匀后，37°C孵育30分钟			
Griess Reagent I	50微升	50微升	50微升
Griess Reagent II	50微升	50微升	50微升
混匀后，室温(20-30°C)孵育10分钟后测定A540			

注意事项：

a. 反应必须避光进行。如果使用96孔板进行检测，可以使用铝箔纸包裹96孔板进行避光。

b. 样品的用量上限为60微升，血清、血浆或组织匀浆液通常使用40微升就足够了。样品不足60微升时，不同样品之间的体积需要保持一致，体积不足的部分用制备或稀释样品时所使用的溶液补足。标准品可以参考上表使用60微升。

c. 可以同时设置加入200微升水或PBS的2-3个孔为阴性对照，这2-3个孔仅仅加入水或PBS，不再加入任何其他试剂。

d. 第一部分37°C孵育30分钟后，直接参考上表依次加入LDH Buffer和LDH，并进行后续孵育。

e. 第二部分37°C孵育30分钟后，直接参考上表依次加入Griess Reagent I和Griess Reagent II。加入Griess Reagent I后需要轻轻混匀。

f. 每次混匀后，可以1000-3000g离心数秒，把液体沉淀到管底。同时需避免各检测孔中产生气泡，以免气泡干扰检测结果。

g. 检测时，如无540nm滤光片，520-560nm的滤光片也可以使用。如无酶标仪或合适的滤光片，也可以通过数码相机拍照后在适当图形软件中进行定量分析，并确定样品中一氧化氮的浓度。拍照比色时标准品需要更为精细的浓度梯度。

5. 根据标准品曲线计算出样品中一氧化氮的浓度。

6. 标准曲线示例见图1，供参考。实际测定时，由于反应条件、试剂盒批次的不同等因素，会导致检测结果与示例数据存在一定差异。

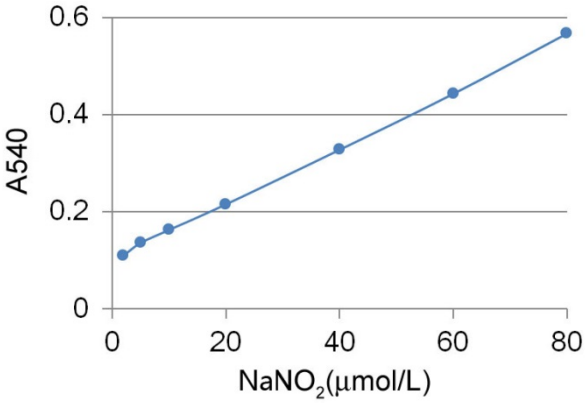

图 1. 亚硝酸钠的参考标准曲线

常见问题：

1. 检测结果标准曲线良好，但样品的吸光度很低，和空白对照接近。

标准曲线良好说明检测方法和检测试剂盒基本上没有问题，样品吸光度低说明样品中一氧化氮含量很低。可以采取的办法是：(1) 加

大样品和标准品的使用量至60微升，其余试剂用量不变。(2) 把整个检测体系每种试剂的用量加大50%，这样可以使检测灵敏度增加约50%。如果上述方法还不能解决问题，可以考虑浓缩样品，即一方面在收集样品的时候尽量使一氧化氮的浓度保持得较高(例如裂解细胞时采用较小体积的裂解液)，另一方面可以考虑用真空干燥或真空冷冻干燥的方法浓缩样品。对于培养的细胞，通常上清液测定出来的吸光度相对较低，而细胞裂解液测定出来的吸光度会稍高一些。

2. 检测时发现每个样品的吸光度都非常高。

在使用RPMI 1640培养液时会发生这种情况。因为RPMI 1640培养液中含有高浓度的硝酸盐从而会使样品检测出来的吸光度都非常高。其他含有高浓度硝酸盐的试剂也会产生类似情况，影响氧化还原反应的试剂也可能产生类似干扰。使用过程中避免使用RPMI 1640等可能导致干扰检测的试剂即可。

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