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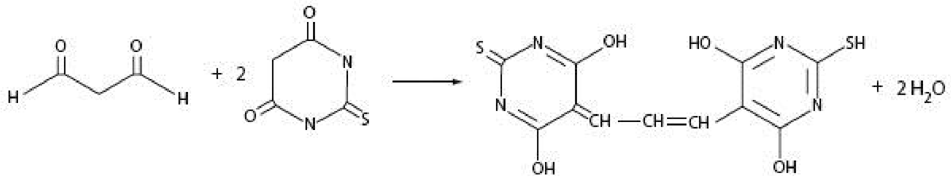
脂质氧化(MDA)检测试剂盒

产品编号	产品名称	包装
S0131	脂质氧化(MDA)检测试剂盒	100次

产品简介：

- 碧云天的脂质氧化(MDA)检测试剂盒(Lipid Peroxidation MDA Assay Kit)采用一种基于MDA和硫代巴比妥酸(thiobarbituric acid, TBA)反应产生红色产物的显色反应，随后通过比色法用于对血浆、血清、尿液、动植物组织或细胞裂解液中MDA进行定量检测，广泛用于脂质氧化(lipid peroxidation) 水平检测的试剂盒。
- 丙二醛(Malondialdehyde, MDA)是一种生物体脂质氧化的天然产物。动物或植物细胞发生氧化应激(oxidative stress)时，会发生脂质氧化。一些脂肪酸氧化后逐渐分解为一系列复杂的化合物，其中包括MDA。此时通过检测MDA的水平即可检测脂质氧化的水平，因此MDA的测定被广泛用作脂质氧化的指标。生物体内的一些其它生化反应也会产生MDA，例如thromboxane synthase也可以催化产生，但只要在测定时设置适当对照即可观察到脂质氧化水平的变化。
- 丙二醛在较高温度及酸性环境中可与TBA发生反应，形成红色的MDA-TBA加合物，相应的反应原理图如下：



MDA TBA MDA-TBA Adduct

- MDA-TBA加合物在535nm处有最大吸收，据此可以通过比色法进行检测。另外，MDA-TBA加合物也可以在535nm被激发产生最大发射波长553nm，据此也可以进行荧光检测。
- **特点：**本试剂盒中采用了特殊的抗氧化剂，可以有效地抑制样品在检测过程中产生新的MDA，使检测更加准确。同时本检测试剂盒在检测过程中可以把部分MDA天然形成的聚丙二醛分解成MDA，使对脂质氧化的测定更加准确。
- 本试剂盒可以检测低达1μM的MDA。血浆、血清样品中的MDA含量通常在约2-4μM，尿液中的MDA含量通常在约5-30μM，在本试剂盒的检测范围内，可以直接用本试剂盒检测血浆、血清、尿液样品等。
- 本试剂盒共可进行100次检测。

包装清单：

产品编号	产品名称	包装
S0131-1	TBA	25mg
S0131-2	TBA配制液	5ml
S0131-3	TBA稀释液	15ml
S0131-4	抗氧化剂	300μl
S0131-5	标准品(1mM)	200μl
—	说明书	1份

保存条件：

-20ºC保存，一年有效。S0131-1 TBA和S0131-4抗氧化剂需避光保存。S0131-1 TBA、S0131-2 TBA配制液和S0131-3 TBA稀释液可室温或4ºC存放三个月。

注意事项：

- 醛、较高浓度的可溶性糖(例如250mM蔗糖)对反应有干扰，可溶性糖与TBA显色反应的产物在532nm也有吸收(最大吸收在450nm)。如果可溶性糖对测定有干扰，可以通过测定450nm作为参考波长进行双波长测定，消除其干扰。
- 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. 样品的准备：

a. 血浆、血清或尿液样品制备后可以直接用于MDA测定。

b. 组织或细胞可以使用PBS或碧云天的Western及IP细胞裂解液(P0013)等裂解液进行匀浆或裂解。对于组织，组织重量占匀

浆液或裂解液的比例为10%；对于细胞，每100万细胞使用0.1ml裂解液或匀浆液。匀浆或裂解后，10,000g-12,000g离心10分钟取上清用于后续测定。对于一些特殊样品，离心不能获得澄清的上清溶液的，可以使用0.2微米孔径的过滤器过滤以获得澄清的样品溶液。匀浆或裂解等样品制备步骤宜在冰浴或4ºC进行操作。

c. 对于组织或细胞样品，样品准备完毕后可以用BCA蛋白浓度测定试剂盒(P0009/P0010/P0010S/P0011/P0012/P0012S)测定蛋白浓度，以便于后续计算单位蛋白重量组织或细胞内的MDA含量。

d. 本试剂盒对于样品中的常见化学成分的兼容性参考下表：

试剂类别	化学成分	是否干扰
缓冲试剂	Borate (50mM)	否
	HEPES (100mM)	否
	Phosphate (100mM)	否
	Tris (25mM)	否
去垢剂	CHAPS (≤1%)	否
	Triton X-100 (≤1%)	否
	Tween 20 (≤1%)	否
抑制剂/螯合剂	Antipain (≤100µg/ml)	否
	Chymostatin (≤10µg/ml)	否
	Leupeptin (≤10µg/ml)	否
	PMSF (≤200µM)	否
	Trypsin (≤10µg/ml)	否
	EDTA (≤1mM)	否
	EGTA (≤1mM)	否
其它试剂	Sucrose (250mM)	是
	Glycerol (≤10%)	否

2. **试剂盒的准备工作：**

a. **TBA储存液的配制：**称取适量TBA，用TBA配制液配制成浓度为0.37%的TBA储存液。例如18.5mg TBA用5ml TBA配制液配制，最终浓度即为0.37%。TBA配制液需完全溶解后再使用，可以加热到70ºC以促进溶解。TBA储存液较难溶解，需加热到70ºC，并通过剧烈Vortex以促进溶解。配制好的TBA储存液室温避光保存，至少3个月内有效。

b. **MDA检测工作液的配制：**根据待测定的样品数(含对照)，参考下表在临检测前新鲜配制适量的MDA检测工作液

检测次数	1次	10次	20次	50次
TBA稀释液	150µl	1500µl	3000µl	7500µl
TBA储存液	50µl	500µl	1000µl	2500µl
抗氧化剂	3µl	30µl	60µl	150µl

注意：MDA检测工作液较难溶解，可以70ºC加热，并剧烈Vortex以促进溶解。也可以通过超声处理以促进溶解。配制好的MDA检测工作液必须当天使用。

c. **标准品的稀释：**取适量标准品用蒸馏水稀释至1、2、5、10、20、50µM，用于后续制作标准曲线。

3. **样品测定:**

a. 在离心管或其它适当容器内加入0.1ml匀浆液、裂解液或PBS等适当溶液作为空白对照，加入0.1ml上述不同浓度标准品用于制作标准曲线，加入0.1ml样品用于测定；随后加入0.2ml MDA检测工作液。可参考下表设置检测反应体系：

	空白对照	标准品	样品
匀浆液、裂解液或PBS	0.1ml	—	—
标准品	—	0.1ml	—
待测样品	—	—	0.1ml
MDA检测工作液	0.2ml	0.2ml	0.2ml

b. 混匀后，100ºC或沸水浴加热15分钟。加热时务必注意避免液体暴沸溅出。如果使用加热块(Heat block)进行加热注意用重物压紧离心管盖；如果使用沸水浴，则需使用可把盖子锁死的离心管或螺旋盖离心管，或用Parafilm封住离心管口，用针头刺一小孔。最方便和准确的加热方法是使用带有热盖并可以加热0.5ml PCR管的PCR仪。

c. 水浴冷却至室温，1000g室温离心10分钟。取200微升上清加入到96孔板中，随后用酶标仪在532nm测定吸光度。如果不方便测定532nm的吸光度，也可以测定530-540nm之间的吸光度。可以设定450nm为参考波长进行双波长测定。

d. MDA含量的计算：对于血浆、血清或尿液等样品可以直接根据标准曲线计算获得MDA的摩尔浓度，对于细胞、或组织样品，计算出样品溶液中的MDA含量后，可以通过单位重量的蛋白含量或组织重量等来表示最初样品中的MDA含量，例如µmol/mg蛋白或µmol/mg组织。

常见问题：

1. 没有检测到MDA。

可能样品中MDA浓度过低，在检测限之下。在检测组织或细胞的MDA时，请注意使用更多的组织或细胞。并注意尽量不要

稀释样品。

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