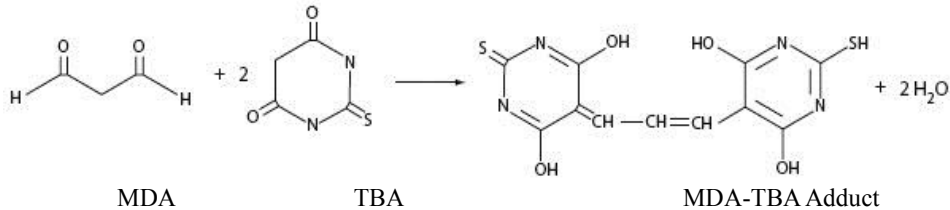


## 脂质氧化(MDA)检测试剂盒

产品编号	产品名称	包装
S0131S	脂质氧化(MDA)检测试剂盒	100次
S0131M	脂质氧化(MDA)检测试剂盒	500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535nm, 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天然形成的聚丙烯二醛分解成MDA, 使对脂质氧化的测定更加准确。
- 本试剂盒可以检测低至1 $\mu$ M的MDA, 也可检测高达200 $\mu$ M的MDA(参考图1)。血浆、血清样品中的MDA含量通常在约2-4 $\mu$ M, 尿液中的MDA含量通常在约5-30 $\mu$ M, 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。

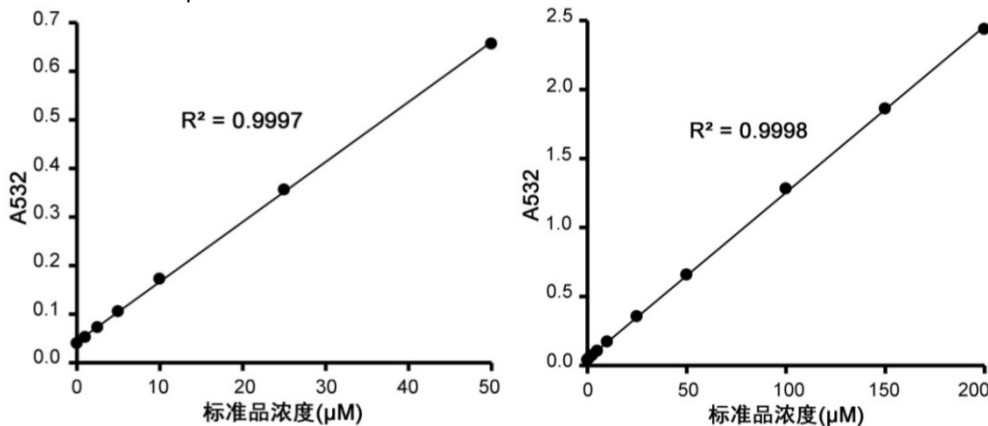


图1. 不同浓度标准品使用本试剂盒的检测效果图。实测数据会因检测仪器等的不同而存在差异, 图中数据仅供参考。

- 本试剂盒S0131S包装共可进行100次检测, S0131M包装共可进行500次检测。

### 包装清单:

产品编号	产品名称	包装
S0131S-1	TBA	25mg
S0131S-2	TBA配制液	6.76ml
S0131S-3	TBA稀释液	15ml
S0131S-4	抗氧化剂	300 $\mu$ l
S0131S-5	标准品(1mM)	200 $\mu$ l

—	说明书	1份
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产品编号	产品名称	包装
S0131M-1	TBA	125mg
S0131M-2	TBA配制液	35ml
S0131M-3	TBA稀释液	75ml
S0131M-4	抗氧化剂	1.5ml
S0131M-5	标准品(1mM)	1ml
—	说明书	1份

### 保存条件：

-20°C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4°C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：

- 血浆、血清或尿液样品制备后可以直接用于MDA测定。
- 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4°C进行操作。
- 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。
- 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 ( $\leq 1\%$ )	否
	Triton X-100 ( $\leq 1\%$ )	否
	Tween 20 ( $\leq 1\%$ )	否
抑制剂/螯合剂	Antipain ( $\leq 100\mu\text{g/ml}$ )	否
	Chymostatin ( $\leq 10\mu\text{g/ml}$ )	否
	Leupeptin ( $\leq 10\mu\text{g/ml}$ )	否
	PMSF ( $\leq 200\mu\text{M}$ )	否
	Trypsin ( $\leq 10\mu\text{g/ml}$ )	否
	EDTA ( $\leq 1\text{mM}$ )	否
其它试剂	EGTA ( $\leq 1\text{mM}$ )	否
	Sucrose (250mM)	是
	Glycerol ( $\leq 10\%$ )	否

#### 2. 试剂盒的准备工作：

- 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，或者25mg TBA用6.76ml 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70°C以促进溶解。TBA储存液较难溶解，需加热到70°C，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。
- 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70°C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。如果样品中MDA的浓度很高, 可以增加100、150和200μM的标准品浓度。

### 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, 100°C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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Version 2019.11.19