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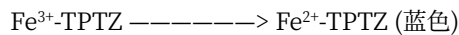
## 总抗氧化能力检测试剂盒(FRAP法)

产品编号	产品名称	包装
S0116	总抗氧化能力检测试剂盒(FRAP法)	100次

### 产品简介:

- 总抗氧化能力检测试剂盒(FRAP法), 即Total Antioxidant Capacity Assay Kit with FRAP method, 简称T-AOC Assay Kit, 是一种采用Ferric Reducing Ability of Plasma (FRAP)方法, 可以对血浆、血清、唾液、尿液等各种体液, 细胞或组织等裂解液、植物或中草药抽提液、或各种抗氧化物(antioxidant)溶液的总抗氧化能力进行检测的试剂盒。
- 活性氧(Reactive oxygen species, ROS)主要包括羟基自由基、超氧自由基和过氧化氢。在细胞或组织的正常生理代谢过程中会产生活性氧, 同时一些环境因子例如紫外照射、 $\gamma$ 射线照射、吸烟、环境污染等也可以诱导活性氧的产生。活性氧产生后, 可以导致细胞内脂、蛋白和DNA等的氧化损伤, 诱发氧化应激(Oxidative stress), 继而导致各种肿瘤、动脉粥样硬化、风湿性关节炎、糖尿病、肝损伤、以及中枢神经系统疾病等。
- 机体中存在多种抗氧化物, 包括抗氧化大分子、抗氧化小分子和酶等, 可以清除体内产生的各种活性氧, 以阻止活性氧诱导的氧化应激(oxidative stress)的产生。一个体系内的各种抗氧化大分子、抗氧化小分子和酶的总的水平即体现了该体系内的总抗氧化能力。因此测定血浆、血清、尿液、唾液等各种体液, 细胞或组织等裂解液中的总抗氧化能力具有非常重要的生物学意义。
- 植物或中草药抽提液、或各种抗氧化物溶液的总抗氧化能力的检测可以用于检测各种溶液的抗氧化能力的强弱, 可以用于筛选强抗氧化能力的药物。
- FRAP法测定总抗氧化能力的原理是酸性条件下抗氧化物可以还原Ferric-tripyridyltriazine ( $Fe^{3+}$ -TPTZ)产生蓝色的 $Fe^{2+}$ -TPTZ, 随后在593nm测定蓝色的 $Fe^{2+}$ -TPTZ即可获得样品中的总抗氧化能力。由于反应在酸性条件下进行, 可以抑制内源性的一些干扰因素。并且由于血浆等样品中的铁离子或亚铁离子的总浓度通常低于 $10\mu M$ , 因此血浆等样品中的铁离子或亚铁离子不会显著干扰FRAP法的检测反应。由于反应体系中的铁离子或亚铁离子是和TPTZ螯合的, 样品本身含有的少量金属离子螯合剂通常也不会显著影响检测反应。

Antioxidant



- 提供了抗氧化物Trolox作为对照。Trolox是一种维生素E的类似物, 水溶性较好, 抗氧化能力和维生素E相近。
- 本试剂盒方便快捷, 加入待测样品后3-5分钟即可进行吸光度测定, 通常10-20个样品可以在十多分钟内检测完毕。
- 本试剂盒可以检测100个样品。

### 包装清单:

产品编号	产品名称	包装
S0116-1	TPTZ稀释液	15ml
S0116-2	TPTZ溶液	1.5ml
S0116-3	检测缓冲液	1.5ml
S0116-4	$FeSO_4 \cdot 7H_2O$	200mg
S0116-5	Trolox溶液 (10mM)	0.1ml
—	说明书	1份

### 保存条件:

-20°C保存, 一年有效。其中S0116-2 TPTZ溶液, S0116-3检测缓冲液和S0116-5 Trolox溶液 (10mM)需避光保存。

### 注意事项:

- 在酸性条件下呈蓝色或接近蓝色的试剂会对本试剂盒的检测产生干扰, 需尽量避免。
- 如果样品中含有外加的较高浓度的铁盐或亚铁盐, 会干扰测定。但血浆、血清、细胞或组织裂解液等样品中含有的微量的铁盐或亚铁盐不会干扰测定。
- 样品中不能添加DTT、巯基乙醇等影响氧化还原反应的物质, 也不宜添加Tween、Triton和NP-40等去垢剂。
- 测定时需可以测定A593的酶标仪一台(测585-605nm也可以)或可以测定微量样品的分光光度计一台。
- TPTZ对人体有刺激性, 操作时请小心, 并注意适当防护以避免直接接触人体或吸入体内。
- 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. FRAP工作液的配制:

- a. 参考下表, 根据待测定样品的数量(含标准曲线)配制适量的FRAP工作液:

	1个检测	5个检测	10个检测	20个检测	50个检测
TPTZ稀释液	150 $\mu$ l	750 $\mu$ l	1500 $\mu$ l	3000 $\mu$ l	7500 $\mu$ l
TPTZ溶液	15 $\mu$ l	75 $\mu$ l	150 $\mu$ l	300 $\mu$ l	750 $\mu$ l
充分混匀后再加入检测缓冲液					
检测缓冲液	15 $\mu$ l	75 $\mu$ l	150 $\mu$ l	300 $\mu$ l	750 $\mu$ l
FRAP工作液	180 $\mu$ l	900 $\mu$ l	1800 $\mu$ l	3600 $\mu$ l	9000 $\mu$ l

FRAP工作液配制后37°C孵育, 并宜在1-2小时内使用完毕。

### 2. 待测样品的准备:

- a. 血清、血浆、唾液或尿液样品的准备:

血清、血浆、唾液或尿液样品每个样品需要5微升, 都可以直接用于测定。血清、血浆、唾液或尿液样品都可以使用新鲜样品进行测定, 也可以-80°C冻存后再进行测定。-80°C冻存的样品至少在一个月内所测定获得的数据没有显著变化。**注意:** 血浆制备时可以使用肝素或柠檬酸钠抗凝, 不宜使用EDTA抗凝。根据文献报道, 人血清或血浆中的总抗氧化能力为0.5-2mM, 人唾液中的总抗氧化能力为0.3-1mM, 人尿液中的总抗氧化能力为0.2-3mM。

- b. 细胞或组织样品的准备:

对于细胞样品, 收集约100万个细胞(不必精确计数, 直接刮下, 不宜使用胰酶和EDTA消化), 放置在200微升冰冷的PBS或HBSS溶液中, 匀浆或超声以充分破碎细胞并释放其中的抗氧化物, 4°C约12000g离心5分钟, 取上清用于后续测定。对于组织样品, 每20mg组织加入100微升冰冷的PBS或HBSS溶液, 匀浆或超声以充分破碎组织并释放其中的抗氧化物, 4°C约12000g离心5分钟, 取上清用于后续测定。以上所有操作均需在4°C或冰上操作。制备好的细胞或组织样品的上清如果不立即用于测定, 可以在-80°C冻存。-80°C冻存的样品至少在一个月内所测定获得的数据没有显著变化。细胞或组织样品在测定总抗氧化能力时需同时测定蛋白浓度, 最后测定获得的总抗氧化能力通常表示为每毫克或每克蛋白重量中的总抗氧化能力, 表示单位为mmol/mg或mmol/g。

- c. 其它样品的准备:

植物或中草药抽提液都可以直接用于检测。需注意样品本身的颜色不会干扰检测。植物或中草药抽提液的抗氧化能力可以表示为每毫克或每克抽提物干重中的总抗氧化能力, 表示单位为mmol/mg或mmol/g。各种抗氧化物测定其抗氧化能力时, 通常配制成0.15-1.5mM, 然后进行测定。抗氧化物的浓度可以以摩尔浓度表示时, 测定获得的总抗氧化能力可以用相对总抗氧化能力进行表示, 例如0.5mM的某抗氧化物其测定获得的OD值和1mM的亚铁盐测定获得的OD值相同, 则其相对总抗氧化能力为2, 再如0.2mM的某抗氧化物其测定获得的OD值和1mM的亚铁盐测定获得的OD值相同, 则其相对总抗氧化能力为5。

### 3. 标准曲线测定的准备:

称取27.8mg本试剂盒提供的  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 溶解并定容到1ml, 此时浓度即为100mM。取适量100mM  $\text{FeSO}_4$ 溶液稀释至0.15、0.3、0.6、0.9、1.2和1.5mM。通常可以使用蒸馏水或样品配制溶液配制标准品。对于血清、血浆、唾液或尿液样品推荐直接用蒸馏水或PBS配制标准品; 对于细胞或组织样品, 推荐用用于细胞或组织匀浆的溶液配制标准品, 其它样品参考前述方法进行。 $\text{FeSO}_4$ 溶液宜新鲜配制使用。100mM  $\text{FeSO}_4$ 溶液容易氧化产生三价铁盐, 使溶液的颜色从最初的淡绿色逐渐转变为浅黄色。如果发现溶液的颜色已经呈现明显的黄色, 应该弃用该溶液, 并使用试剂盒提供的  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 重新配制新鲜的  $\text{FeSO}_4$ 溶液。

### 4. 总抗氧化能力的测定:

- a. 96孔板的每个检测孔中加入180微升FRAP工作液。  
b. 空白对照孔中加入5微升蒸馏水或PBS等适当溶液; 标准曲线检测孔内加入5微升各种浓度的  $\text{FeSO}_4$ 标准溶液; 样品检测孔内加入5微升各种样品或0.15-1.5mM的Trolox作为阳性对照。轻轻混匀。  
c. 37°C孵育3-5分钟后测定A593。如果测定A593有困难, 也可以在585-605nm范围内进行测定。  
d. 根据标准曲线计算出样品的总抗氧化能力。如果样品测定出来的吸光度在标准曲线范围以外, 需把样品适当稀释后再进行测定。  
e. **总抗氧化能力的表示方式:** 对于FRAP方法, 总抗氧化能力用  $\text{FeSO}_4$ 标准溶液的浓度来表示。例如某血浆样品测定获得的吸光度和1mM  $\text{FeSO}_4$ 标准溶液的吸光度相同, 则该血浆样品的总抗氧化能力即为1mM; 再如某血清样品测定获得的吸光度和0.65mM  $\text{FeSO}_4$ 标准溶液相同, 则该血清样品的总抗氧化能力为0.65mM; 再如某细胞匀浆液测定获得的吸光度和0.3mM  $\text{FeSO}_4$ 标准溶液相同, 并且该匀浆液的蛋白浓度为0.15mg/ml, 则该细胞样品的总抗氧化能力为0.3mM/0.15mg/ml, 即2mmol/g; 再如0.2mM的某抗氧化物其测定获得的吸光度和1mM的亚铁盐测定获得的吸光度相同, 则其相对总抗氧化能力为5。

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