碧云天生物技术/Beyotime Biotechnology
订货热线: 400-1683301 或 800-8283301
订货 e-mail: order@beyotime.com
技术咨询: info@beyotime.com
网址: http://www.beyotime.com

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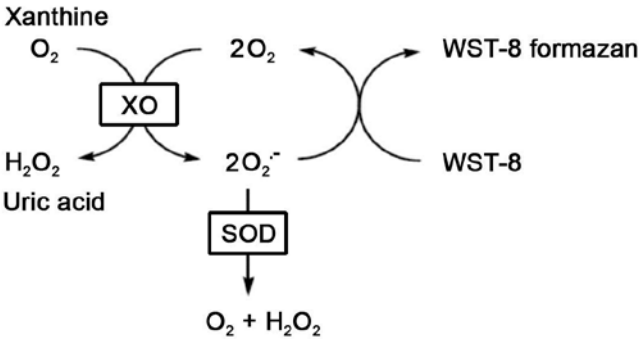
CuZn/Mn-SOD活性检测试剂盒(WST-8法)

产品编号	产品名称	包装
S0103	CuZn/Mn-SOD活性检测试剂盒(WST-8法)	100次

产品简介：

- 碧云天的CuZn/Mn-SOD活性检测试剂盒(WST-8法)(Cu/Zn-SOD and Mn-SOD Assay Kit with WST-8)是一种基于WST-8的显色反应，通过比色来检测细胞、组织或其它样品中Cu/Zn-SOD、Mn-SOD或总SOD活性的试剂盒。
- 超氧化物岐化酶(Superoxide Dismutase, SOD)能催化超氧化物阴离子发生岐化作用，生成过氧化氢(H_2O_2)和氧气(O_2)，是生物体内一种重要的抗氧化酶。
- 目前有多种SOD活性测定法，其中NBT(氮蓝四唑)法由于使用方便而被广泛使用。但NBT法产生的甲臜染料水溶性差，易和被还原的黄嘌呤氧化酶相互作用，抑制百分率达不到100%等，从而使检测的灵敏度和精确度受到影响；细胞色素C法也是一种常用来检测SOD活性的方法，但细胞色素C其氧化活性高，易受样品中的还原剂干扰，另外该方法需要连续测定吸光度值，对于SOD的检测灵敏度比较低，并且不太适合大批量样本的检测。
- 目前测定SOD比较先进的方法包括WST-1法和WST-8法，其中WST-8法比WST-1法更加稳定、灵敏度更高。本试剂盒采用了目前测定SOD方法中稳定性更好、灵敏度更高的的WST-8法。WST-8法的原理参考下图，WST-8可以和黄嘌呤氧化酶催化产生的超氧化物阴离子反应产生水溶性的甲臜染料(formazan dye)，该反应步骤可以被SOD所抑制。通过对WST-8产物的比色分析即可计算SOD的酶活力。



基于黄嘌呤氧化酶藕联反应体系和WST-8的SOD酶活力检测原理图。XO：xanthine oxidase。

- WST-8的反应产物是稳定的水溶性产物，可以通过单个时间点的吸光度检测来测定SOD活力，适合高通量筛选研究。同时WST-8法测定SOD酶活力时，最大抑制百分率可以接近100%，并且可以不受一些常见的干扰因素的干扰，使检测效果比其它的一些常见方法显著改善。
- 本试剂盒的检测不受样品中过氧化氢的干扰。很多细胞和组织样品中含有内源性的过氧化氢，会干扰SOD的检测。本试剂盒通过添加适量过氧化氢酶等特殊方法，能有效去除常规样品中过氧化氢的干扰。例如，对于SOD标准品的检测，标准品中添加高达0.1mM的过氧化氢时，对于检测结果仍无显著影响。
- 哺乳动物编码3种SOD。在大部分组织中，含量最高的是定位在细胞浆中的Cu/Zn-SOD。Mn-SOD主要表达在线粒体中，并且Mn-SOD是可以被诱导表达的。Mn-SOD在某些情况下也可以定位在细胞浆中。有些组织或体液中会含有第三种SOD，extracellular SOD，简称EC-SOD。EC-SOD也是一种Cu/Zn-SOD，和编码细胞浆Cu/Zn-SOD的基因相似性很高，但有所不同。综上所述，细胞或组织内共有两类SOD，即铜锌超氧化物歧化酶(Cu/Zn-SOD)和锰超氧化物歧化酶(Mn-SOD)。这两种SOD酶活性的总量就是总SOD的酶活性。当用Cu/Zn-SOD的抑制剂抑制其酶活性时测定得到的就是Mn-SOD的酶活性。如果此时同时测定总的SOD酶活性，就可以计算出Cu/Zn-SOD的酶活性。
- KCN或NaCN可抑制CuZn-SOD酶活性，也被大量用于测定Mn-SOD的酶活性。但KCN或NaCN都具有剧毒，并且对Cu/Zn-SOD的抑制百分比达不到很接近100%。
- 本试剂盒采用了一种无毒的两步双重抑制Cu/Zn-SOD的方法，在避免使用有毒药品的情况下同时确保了对于Cu/Zn-SOD的抑制百分比达到高度接近100%，即对于Cu/Zn-SOD的抑制效果显著优于KCN或NaCN。使对于Cu/Zn-SOD或Mn-SOD酶活力的测定更加安全和准确。
- 本试剂盒可以检测细胞或组织匀浆液上清、全血、红细胞抽提物、血清等生物样品中的SOD活性。一个试剂盒共可以进行100次检测。

包装清单：

产品编号	产品名称	包装
S0103-1	SOD检测缓冲液	70ml
S0103-2	WST-8	800μl
S0103-3	酶溶液	100μl
S0103-4	反应启动液(40X)	60μl
S0103-5	Cu/Zn-SOD抑制剂A	500μl
S0103-6	Cu/Zn-SOD抑制剂B	500μl
—	说明书	1份

保存条件：

-20°C保存，半年有效。S0103-2 WST-8溶液需避光保存。

注意事项：

- 待测样品-70°C可保存1个月。需注意反复冻融会导致SOD部分失活。
- 细胞或组织等样品制备时不能采用含有Triton X-100等去垢剂的溶液，否则会干扰本试剂盒的检测。
- 抗氧化物会对本试剂盒的检测产生干扰，例如0.1mM ascorbic acid，5mM GSH都会使测定出来的吸光度显著升高。此时尽管样品没有颜色，如果设置了使用说明中的空白对照3，就可以消除样品中的抗氧化物的干扰。
- Cu/Zn-SOD抑制剂A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．样品的准备：

a．**细胞样品的准备：**收集细胞，用4°C或冰浴预冷的PBS或生理盐水洗涤1-2遍。沉淀用预冷的PBS在4°C或冰浴进行匀浆(可以使用玻璃匀浆器或各类常见电动匀浆器)。随后匀浆液4°C离心，取上清作为待测样品。

b．**组织样品的准备：**动物用生理盐水(0.9% NaCl，含有0.16mg/ml肝素钠)灌流清除血液后获取组织样品。取适量的组织样品，加入4°C或冰浴预冷的PBS在4°C或冰浴进行匀浆(可以使用玻璃匀浆器或各类常见电动匀浆器)。随后匀浆液4°C离心，取上清作为待测样品。

c．**血浆或红细胞样品的准备：**用抗凝管收集血液，颠倒混匀。4°C 600g离心10分钟，移取上清至另一新的1ml离心管中，适量生理盐水稀释后即可作为血浆样本进行检测。红细胞样品可以参考步骤1a细胞样品的制备方法，或其它不含Triton X-100等去垢剂的样品制备方法。

d．上述样品准备完毕后可以用碧云天生产的BCA蛋白浓度测定试剂盒(P0009/P0010/P0010S/P0011/P0012/P0012S)测定蛋白浓度。通常10-20微克蛋白的细胞或组织匀浆液样品其中的SOD平均活力约1个活力单位左右(不同细胞和组织的差异会比较大，该活力范围仅作为初步的参考)。每种样品准备20-100微克蛋白量通常已经足够用于后续检测。

e．根据蛋白浓度和预计的蛋白使用量，用本试剂盒提供的SOD检测缓冲液适当稀释样品。例如，小鼠肝脏组织10%匀浆液(组织和匀浆液的重量比为10%)上清，通常需要稀释10-100倍。准备好的样品如果当天测定，可以冰浴保存；如果当天不能完成测定，可以-70°C冻存，但建议尽量当天完成测定。

f．Cu/Zn-SOD的抑制(测定Mn-SOD或Cu/Zn-SOD时选做)：将Cu/Zn-SOD抑制剂A和经适当稀释的样品或标准品按1:24的体积比(如4μl +96μl)，在离心管内或96孔板中混合好，37°C孵育1小时；取20微升Cu/Zn-SOD抑制剂B加入到780微升水中，混匀，即进行40倍稀释。然后将已经40倍稀释的Cu/Zn-SOD抑制剂B和上述混合物再按1:25的体积比混合均匀(如4μl+100μl)，37°C再孵育15分钟。随后即可进行步骤3a，或暂时4度或冰浴保存。样品处理后宜当日使用，并尽量在处理后尽快进行后续检测。注1：稀释后的Cu/Zn-SOD抑制剂B宜当天使用，多余的已稀释的Cu/Zn-SOD抑制剂B可以直接丢弃。注2：本试剂盒中提供的Cu/Zn-SOD抑制剂A和抑制剂B用于待测样品中Cu/Zn-SOD酶活性的抑制；不宜用于添加到培养的细胞、组织中，或注射到活体动物中以抑制Cu/Zn-SOD。

2．试剂盒的准备工作：

a．**WST-8/酶工作液的配制：**按照每个反应160μl的体积配制适量的WST-8/酶工作液。均匀混合151μl SOD检测缓冲液、8μl WST-8和1μl酶溶液，即可配制成160μl WST-8/酶工作液。根据待检测样品(包括标准品)的数量，配制适量的WST-8/酶工作液。具体配制方法可以参考下表。配制好的WST-8/酶工作液4°C或冰浴保存，可以在当天使用，但建议尽量现配现用。**注意：**由于酶溶液的用量较少且易沉降，必须注意在使用前先轻轻离心一下，然后适当混匀后再使用。

待测样品数量	1	10	20	50
SOD检测缓冲液(μl)	151	1510	3020	7550
WST-8(μl)	8	80	160	400
酶溶液(μl)	1	10	20	50
WST-8/酶工作液(μl)	160	1600	3200	8000

b．**反应启动工作液的配制：**把试剂盒中的反应启动液(40X)融解后混匀，按照每1μl反应启动液(40X)加入39μl SOD检测缓冲液的比例进行稀释，混匀后即为反应启动工作液。根据待检测样品(包括标准品)的数量，配制适量的反应启动工作液。配制好的反应启动工作液4°C或冰浴保存，可以在当天使用，但建议尽量现配现用。

c. (可选做)SOD标准品准备：需自备SOD标准品，用本试剂盒提供的稀释液将SOD标准品稀释至如下系列浓度：200U/ml，100U/ml，50U/ml，20U/ml，10U/ml，5U/ml，2U/ml。在随后的检测中可以各取20微升，参考样品进行检测。**说明：**为避免稀释后SOD酶活性的下降，SOD标准品宜现稀释现使用；本试剂盒对于SOD的检测并不需要SOD作为标准品，但可以使用SOD标准品作为阳性对照或作为对SOD活性定量的参考。

3. 样品测定：

a. 参考下表使用96孔板设置样品孔和各种空白对照孔。并按下表依次加入待测样品和其它各种溶液。加入反应启动工作液后充分混匀。**注意：**加入反应启动工作液后反应即会开始，可以在低温操作或用排枪操作以减小各孔间因加入反应启动工作液的时间先后差异而导致的误差。

	样品(Sample)	空白对照1(Blank1)	空白对照2(Blank2)	空白对照3(Blank3)*
待测样品	20μl	—	—	20μl
SOD检测缓冲液	—	20μl	40μl	20μl
WST-8/酶工作液	160μl	160μl	160μl	160μl
反应启动工作液	20μl	20μl	—	—

*如果样品有颜色或含有抗氧化物质，则需设置空白对照3；如果样品没有颜色并且也不含有抗氧化物则没有必要设置空白对照3。

b. 37°C孵育30分钟。**说明：**孵育25至35分钟检测出来的SOD活力无显著差异，但为保证检测结果的一致性，推荐孵育30分钟。

c. 在450nm测定吸光度。如无450nm滤光片，可以使用420-480nm的滤光片。可以使用大于600nm的波长作为参考波长进行双波长测定。

4. 样品中总SOD活力的计算：

a. 抑制百分率的计算：

参考如下计算公式计算抑制百分率：

抑制百分率 ＝ [($A_{空白对照1}$－$A_{空白对照2}$)－($A_{样品}$－$A_{空白对照3}$)] / ($A_{空白对照1}$－$A_{空白对照2}$) × 100%

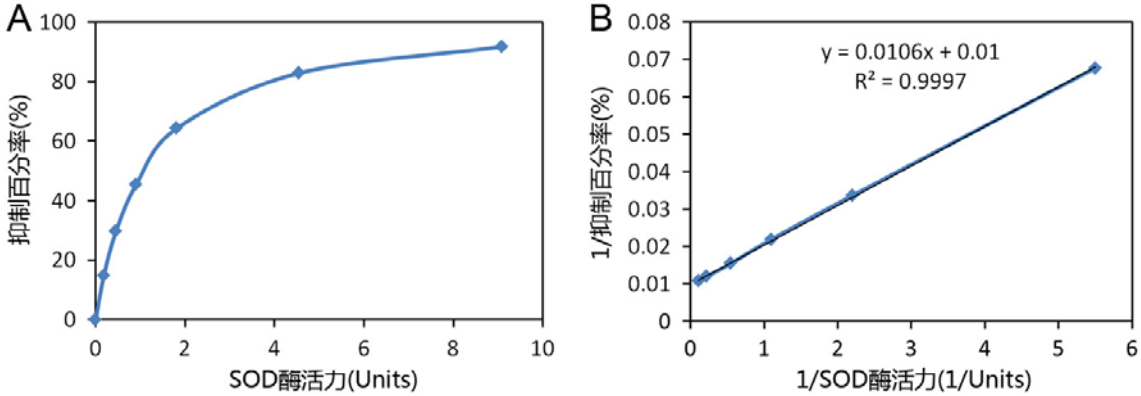
如果没有设置空白对照3，则可以把计算公式简化为：

抑制百分率 ＝ ($A_{空白对照1}$－$A_{样品}$) / ($A_{空白对照1}$－$A_{空白对照2}$) × 100%

如果计算出来的抑制百分率小于30%或大于70%，则通常需要把该样品重新测定。尽量使样品的抑制百分率在30-70%范围内。如果测定出来的抑制百分率偏高，则需适当稀释样品；如果测定出来的抑制百分率偏低，则需重新准备浓度较高的待测样品。

b. SOD酶活力单位的定义：在上述黄嘌呤氧化酶藕联反应体系中抑制百分率为50%时，反应体系中的SOD酶活力定义为一个酶活力单位(unit)。注意：SOD的活力单位的定义方式有很多种，不同的活力单位需根据其定义的不同进行适当换算。

c. SOD酶活力的计算：



参考上图A和B，SOD酶活力和抑制百分率呈非线性关系，而1/SOD酶活力和1/抑制百分率成线性关系。上图仅作参考，不同的样品不同的检测条件，实际测定获得的标准曲线的斜率和截距可能和上图有较明显的差别。

SOD酶活力的计算公式如下：

待测样品中SOD酶活力单位＝检测体系中SOD酶活力单位＝抑制百分率 / (1－抑制百分率) units

例如，当抑制百分率为50%时，待测样品中SOD酶活力单位＝50% / (1－50%)units＝1 unit；当抑制百分率为60%时，待测样品中SOD酶活力单位＝60% / (1－60%)units＝1.5 units。

d. 如果样品为细胞或组织的匀浆液，可以根据样品的蛋白浓度和稀释倍数，将SOD活力单位换算为U/g或U/mg蛋白。如果样品为红细胞抽提液，可以根据血红蛋白含量，可换算为U/克血红蛋白或U/毫克血红蛋白。

附1：SOD酶活力计算的参考方案：可以先使用本试剂盒绘制SOD标准品的抑制百分率曲线，然后根据样品检测到的抑制百分率对比标准品的抑制百分率曲线计算出样品中的SOD酶活力单位。本方案仅供参考，使用本试剂盒时不必使用本方案进行检测和计算。此外，本方案需确保标准品的酶活力数据可靠，不会因为标准品的保存问题而导致实际酶活力下降。

附2：SOD酶活力的动力学检测：如果条件许可，使用本试剂盒时也可以使用动力学方法检测SOD的酶活力。通常在步骤3a后可以37°C孵育同时在450nm连续测定吸光度30分钟。根据30分钟内的吸光度变化的斜率计算出抑制百分率：

抑制百分率 ＝ [($斜率_{空白对照1}$－$斜率_{空白对照2}$)－($斜率_{样品}$－$斜率_{空白对照3}$)] / ($斜率_{空白对照1}$－$斜率_{空白对照2}$)× 100%

其余的计算方法同上述非动力学的计算方法。动力学方法的检测和计算更加精确一些，但检测起来相对要麻烦一些。使用本试剂盒通常使用非动力学方法即可。

使用本产品的文献：

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