



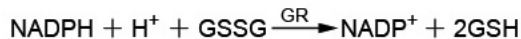
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## GSH和GSSG检测试剂盒

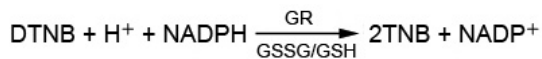
产品编号	产品名称	包装
S0053	GSH和GSSG检测试剂盒	共100次

### 产品简介:

- GSH和GSSG检测试剂盒(GSH and GSSG Assay Kit)是一种简单易行的可以分别检测出GSH(还原型谷胱甘肽)和GSSG(氧化型谷胱甘肽, oxidized glutathione disulfide)含量的检测试剂盒。
- 谷胱甘肽(glutathione)是一种由3个氨基酸残基组成的小肽, 全称为谷氨酰-半胱氨酰-甘氨酸, 英文名称为glutamyl-cysteinyl-glycine, 简称为glutathione。由于半胱氨酸上的巯基(SH)为谷胱甘肽的活性基团, 所以常简称为G-SH或GSH。谷胱甘肽包括还原型谷胱甘肽(reduced glutathione, 常称为GSH)和氧化型谷胱甘肽(oxidized glutathione disulfide)两种形式。由于氧化型谷胱甘肽是由两个GSH通过巯基脱氢而成, 所以常简称为G-S-S-G或GSSG。还原型谷胱甘肽是绝大多数活细胞中巯基的主要来源, 对于维持蛋白质中巯基适当的氧化还原状态有重要作用, 并且是动物细胞中关键的抗氧化剂。总谷胱甘肽中通常90-95%为还原型谷胱甘肽。
- 通过谷胱甘肽还原酶把GSSG还原成GSH, 而GSH可以和生色底物DTNB反应产生黄色的TNB和GSSG。适当配制反应体系, 前后两个反应合并起来后, 总谷胱甘肽(GSSG+GSH)就相当于一个颜色产生的限速因素, 总谷胱甘肽的量就决定了黄色的TNB形成量。从而通过测定A<sub>412</sub>就可以计算出总谷胱甘肽的量。用适当试剂先清除样品中的GSH, 然后利用上述反应原理就可以测定出GSSG的含量。用总谷胱甘肽(GSSG+GSH)的量扣除GSSG的含量, 就可以计算出GSH的含量。
- 本试剂盒的具体反应原理如下:



两个反应相合并:



- 本试剂盒可以检测动物组织、血浆、红细胞、和培养细胞或其它适当样品中GSH和GSSG的含量。
- 本试剂盒提供了蛋白去除试剂M, 可以更加准确地测定出含有蛋白的样品中的GSH和GSSG的量。
- 本试剂盒的检测下限为0.5μM。一个试剂盒共可以进行100次检测, 可以测定100个样品的总谷胱甘肽或GSSG的含量, 或可以测定出50个样品中GSH和GSSG的各自含量。

### 包装清单:

产品编号	产品名称	包装
S0053-1	总谷胱甘肽检测缓冲液	60ml
S0053-2	谷胱甘肽还原酶	150μl
S0053-3	氧化型谷胱甘肽 (GSSG)	5mg
S0053-4	DTNB	4.5mg
S0053-5	蛋白去除试剂M	1g
S0053-6	NADPH	4mg
S0053-7	DMSO	1.5ml
S0053-8	GSH清除辅助液	2ml
S0053-9	GSH清除试剂	500μl
—	说明书	1份

### 保存条件:

-20°C保存, 一年有效。GSSG配制成溶液后, 需适当分装, -20°C保存至少3个月有效。DTNB溶解于DMSO后, 需适当分装, -20°C保存至少3个月有效。蛋白去除试剂M配制成溶液后仅限当天使用。NADPH溶解后, 适当分装, -70°C保存。稀释的GSH清除辅助液和GSH清除试剂溶液均须新鲜配制使用。

### 注意事项:

- 本试剂盒检测时牵涉到氧化还原反应, 所有氧化剂或还原剂都会干扰本试剂盒的测定。特别是DTT、巯基乙醇等含有巯基的试剂会严重干扰本试剂盒的测定, 请尽量避免。

- 一定要严格控制反应时的温度和反应时间，否则每次都需做标准曲线。
- NADPH等试剂不太稳定，要严格按照后续说明操作，谨防失活。
- 蛋白去除试剂M溶液必须新鲜配制并限当日使用。GSH清除试剂也须新鲜稀释后使用。
- 蛋白去除试剂M较难溶解，可以通过剧烈vortex并适当加热(不超过37°C)以促进溶解。
- DMSO在4°C、冰浴等较低温度情况下会凝固，可以20-25°C水浴温育片刻至全部融解后使用。
- 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. 试剂盒的准备工作：

- GSSG储备液的配制：在本试剂盒提供的5mg GSSG中加入816微升Milli-Q级纯水，溶解并混匀，即为GSSG储备液，浓度为10mM。除立即待用部分外，其余GSSG储备液适当分装后-20°C保存。
- DTNB储备液的配制：在本试剂盒提供的4.5mg DTNB中加入1.5毫升本试剂盒提供的DMSO，溶解并混匀，即为DTNB储备液。除立即待用部分外，其余DTNB储备液适当分装后-20°C保存。
- 蛋白去除试剂M溶液的配制：称取0.2克蛋白去除试剂M，加入4毫升总谷胱甘肽检测缓冲液，配制成4毫升5%的水溶液。蛋白去除试剂M溶液必须新鲜配制并限当天使用。
- NADPH储备液(40mg/ml)的配制：在本试剂盒提供的4mg NADPH中加入100微升Milli-Q级纯水，溶解并混匀，即为NADPH储备液。除立即待用部分外，其余NADPH储备液适当分装后-70°C保存。
- 5倍稀释谷胱甘肽还原酶的配制：取50微升谷胱甘肽还原酶，加入200微升总谷胱甘肽检测缓冲液，混匀，即成5倍稀释的谷胱甘肽还原酶。
- 总谷胱甘肽检测工作液的配制：根据待检测的样品数参考下表配制适当量的总谷胱甘肽检测工作液，表中三种试剂按比例混合后即总谷胱甘肽检测工作液。

	1个样品	10个样品	20个样品
5倍稀释谷胱甘肽还原酶	6.6 μl	66 μl	132 μl
DTNB储备液	6.6 μl	66 μl	132 μl
总谷胱甘肽检测缓冲液	150 μl	1.5 ml	3 ml

- 0.5mg/ml NADPH的配制：取10微升NADPH储备液，加入790微升总谷胱甘肽检测缓冲液，混匀即为0.5mg/ml NADPH。每检测一个样品需50微升0.5mg/ml NADPH。
- 稀释的GSH清除辅助液的配制：在47微升Milli-Q级纯水中加入53微升GSH清除辅助液，立即混匀。稀释后的GSH清除辅助液不太稳定，每次使用时均须新鲜配制，并仅限当日使用。
- GSH清除试剂工作液的配制：10.8微升GSH清除试剂中加入89.2微升无水乙醇，立即混匀。GSH清除剂工作液每次也须新鲜配制。

### 2. 标准品的准备：

- 把10mM GSSG储备液用蛋白去除试剂M溶液稀释成15μM GSSG溶液。然后依次稀释成10、5、2、1、0.5μM GSSG溶液。取15、10、5、2、1、0.5μM GSSG溶液六个点做标准曲线。注意：由于GSSG在蛋白去除试剂M溶液中不太稳定，用蛋白去除试剂M溶液配制的GSSG溶液必须新鲜配制后使用，不可冻存后再使用。
- 如果需要测定样品中GSSG含量，按照每100微升标准品加入20微升稀释的GSH清除辅助液的比例加入稀释的GSH清除辅助液，立即vortex混匀。然后按照每100微升加入4微升GSH清除试剂工作液的比例加入GSH清除试剂工作液，立即vortex混匀，25°C反应60分钟。即可用于后续的GSSG含量的检测。

### 3. 待测总谷胱甘肽含量样品和标准品的准备：

- 组织样品的准备。**取组织用液氮速冻，然后研成粉末。每10毫克研碎的组织粉末，加入30微升蛋白去除试剂M溶液，充分Vortex。再加入70微升蛋白去除试剂M溶液，用玻璃匀浆器充分匀浆(对于比较容易匀浆的组织可以不用液氮速冻等处理，而直接加入适量蛋白去除试剂M溶液进行匀浆)。4°C放置10分钟后，10,000g 4°C离心10分钟，取上清用于总谷胱甘肽的测定。样品需暂时4°C保存，不立即测定的样品可以-70°C保存，但不宜超过10天。对于处理好的组织样品通常需用蛋白去除试剂M溶液进行适当稀释后再进行测定，稀释倍数通常为5-20倍。
- 细胞样品的准备。**请尽量使用新鲜的细胞进行测定，而不要使用冻存的细胞进行测定。PBS洗涤细胞一次，离心收集细胞，吸尽上清。加入细胞沉淀体积3倍量的蛋白去除试剂M溶液，即如果细胞沉淀为10微升，则加入30微升蛋白去除试剂M溶液，充分Vortex。(细胞沉淀的体积可以根据细胞沉淀的重量进行估算。收集细胞前后分别对离心管进行称重，从而就可以计算出细胞沉淀的重量。10毫克细胞沉淀的体积可以粗略地看做10微升。)然后利用液氮和37°C水浴对样品进行两次快速的冻融。4°C或冰浴放置5分钟。4°C，10,000g离心10分钟。取上清用于总谷胱甘肽的测定。样品需暂时4°C保存，不立即测定的样品可以-70°C保存，但不宜超过10天。对于处理好的细胞样品通常需用蛋白去除试剂M溶液进行适当稀释后再进行测定，稀释倍数可以高达20倍。
- 红细胞或血浆样品的准备。**请尽量使用新鲜的血液进行测定。600g离心10分钟，沉淀为红细胞，上清为血浆。对于红细胞，用PBS洗涤两次。取约50微升红细胞沉淀或血浆，加入50微升蛋白去除试剂M溶液，充分Vortex。4°C或冰浴放置10分钟。4°C，10,000g离心10分钟。取上清用于总谷胱甘肽的测定。样品需暂时4°C保存，不立即测定的样品可以-70°C保存，但不宜超过10天。对于处理好的红细胞样品最后需用蛋白去除试剂M溶液稀释10倍后再进行后续的测定，而对于血浆样品，应直接取10微升进行测定。

d. **说明：**对于一些谷胱甘肽含量特别低的样品，可以通过冷冻干燥进行浓缩后再进行测定。

#### 4. 待测GSSG含量样品的准备：

取部分上述准备好的待测总谷胱甘肽含量的样品，按照每100微升样品加入20微升稀释的GSH清除辅助液的比例加入稀释的GSH清除辅助液，立即vortex混匀。再按照每100微升样品加入4微升GSH清除试剂工作液的比例加入GSH清除工作液，立即vortex混匀，25°C反应60分钟。通过上述反应可以清除高达50μM的GSH，如果样品中GSH含量过高需进行适当稀释后再进行去除GSH的操作。通过上述处理就可以用于后续的测定。

#### 5. 样品和标准品的测定：

a. 参考下表，使用96孔板，依次加入样品或标准品，混匀。加入150微升总谷胱甘肽检测工作液后，混匀，25°C或室温孵育5分钟。

	空白对照 (blank)	标准曲线 (standard)	样品(sample)
样品或标准品	0 μl	10 μl	x μl
蛋白去除试剂M溶液	10 μl	0 μl	10 - x μl
总谷胱甘肽检测工作液	150 μl	150 μl	150 μl
25°C或室温孵育	5 min	5 min	5 min
0.5mg/ml NADPH	50 μl	50 μl	50 μl

b. 加入50微升0.5mg/ml NADPH溶液，混匀。

c. 立即用酶标仪测定A<sub>412</sub>，每5分钟测定一次或实时测定，共测定25分钟，测得5个数据。(说明：为了简化实验步骤，可以在加入NADPH溶液混匀后25分钟，仅测定一次A<sub>412</sub>)。如果仪器可以设置温度，把温度设置在25°C，否则就在室温状况下测定。如果酶标仪不能测定A<sub>412</sub>，可以测定405-414nm附近范围的吸光度。如果标准曲线良好，但样品的吸光度比较低，可以延长孵育时间至30-60分钟，标准品和样品的吸光度在一定范围内会随时间的延长接近于线性增加的。

**注意：**如果进行GSSG含量测定，标准品也须平行地进行去除GSH的相关操作，以减小误差。如果样品需同时测定总谷胱甘肽含量和GSSG含量，由于两者的检测体系不同，须分别单独做标准曲线。

d. 标准品的实测效果参考图1。

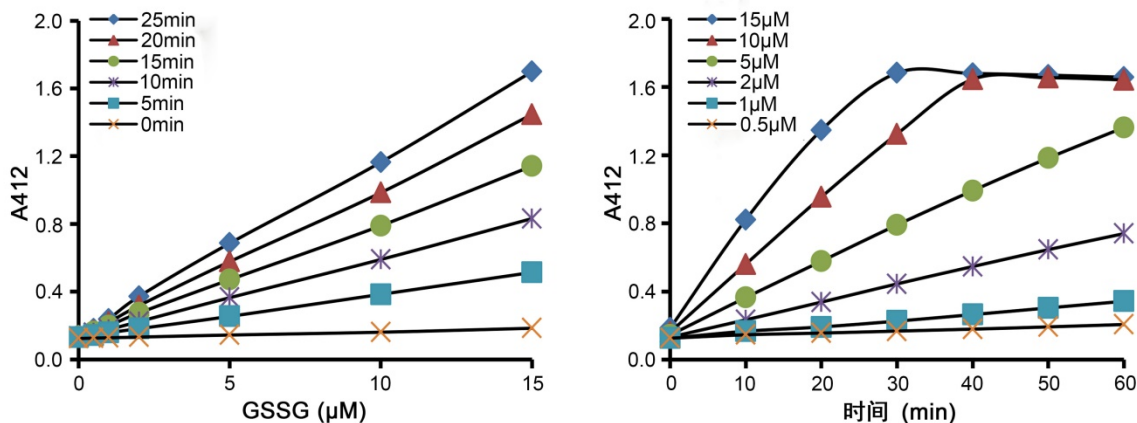


图1. GSSG标准曲线实测效果图。图中数据仅供参考，实际的检测效果可能会因具体反应条件的不同而有所不同。

#### 6. 样品中总谷胱甘肽含量的计算：

a. **单点测定法：**即反应25分钟(或30-60分钟)后仅测定一次吸光度。根据不同浓度标准品测得的不同吸光度作出标准曲线。样品对照标准曲线即可计算出总谷胱甘肽(标准曲线计算得到的GSSG浓度乘以2)或GSSG的含量。实际计算出来的总谷胱甘肽的含量相当于把氧化型谷胱甘肽的含量乘以2再加上还原型谷胱甘肽的含量。单点法测定相对比较便捷，而动力学法测定则相对比较精确。注意：由于1个GSSG分子反应后可以还原成2个GSH分子，所以GSSG的浓度如果换算成GSH的浓度时需乘以2，例如完全清除样品中内源GSH的情况下，GSSG的浓度为5μM，则相当于GSH的浓度为10μM。

b. **动力学测定法：**先根据不同时间点测定得到的吸光度值计算出 $\Delta A_{412}/\text{min}$ 。然后以标准品的浓度为横坐标，以 $\Delta A_{412}/\text{min}$ 为纵坐标，做出标准曲线。根据样品的 $\Delta A_{412}/\text{min}$ ，对照标准曲线就可以计算出测定时样品中总谷胱甘肽或GSSG的含量。

c. 同时根据样品的稀释倍数以及最初样品的使用量，可以计算出每毫克组织或细胞中的总谷胱甘肽或GSSG的含量。对于细胞样品，也可以根据最初细胞的使用数量，然后另外取一定数量的细胞裂解后测定蛋白浓度，从而计算出细胞样品的蛋白量，最后计算出每毫克蛋白中总谷胱甘肽或GSSG的含量。

d. 根据测定得到的总谷胱甘肽的含量和GSSG的含量就可以计算出GSH的含量。

计算公式为： $\text{GSH} = \text{Total Glutathione} - \text{GSSG} \times 2$  (注意：Total Glutathione为通过标准曲线计算得到的GSSG浓度乘以2，同时清除GSH后得到的GSSG也要乘以2，因为1个GSSG分子在反应后可以还原成2个GSH分子)。例如通过本试剂盒测定的总谷胱甘肽(Total Glutathione)的浓度是15μM (即在测定总谷胱甘肽时通过标准曲线计算得到的GSSG浓度为7.5μM，乘以2即为总谷胱甘肽浓度)，测定的GSSG的浓度是1.2μM (即在单独测定GSSG含量时通过标准曲线计算得到的GSSG浓度为1.2μM)，那么样品中GSH的浓度为 $15 - 1.2 \times 2 = 12.6 \mu\text{M}$ 。

## 相关产品:

产品编号	产品名称	包装
S0052	总谷胱甘肽检测试剂盒	100次
S0052B	蛋白去除试剂S	3g
S0053	GSH和GSSG检测试剂盒	共100次
S0054	蛋白去除试剂M	5g
S0055	谷胱甘肽还原酶检测试剂盒	100次
S0056	谷胱甘肽过氧化物酶检测试剂盒	100次
S0058	总谷胱甘肽过氧化物酶检测试剂盒	100次

## 使用本产品的文献:

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