本试剂盒仅供体外研究使用，不用于临床诊断！

FineTest®

GSH（还原型谷胱甘肽）比色法检测试剂盒

GSH (Reduced Glutathione) colorimetric Assay Kit

产品货号：EU2640

版本号: V4.0

包装规格：48T/96T

请勿将不同货号、不同批次号的试剂混用，否则试剂盒将无法正常工作

使用前请仔细阅读说明书。如果有任何问题，可通过以下方式联系我们：

销售部电话　027-86697005

技术部电话　18064071591（ELISA 售后）

技术部电话　18107218793（ELISA 售前）

电子邮箱　sales2@fn-test.com

网　　址　https://www.fn-test.cn/

复购时请提供产品批号(见试剂盒标签)，以便我们更高效地为您服务。

具体保质期请见试剂盒外包装标签。请在保质期内使用试剂盒。

如有更大包装需求可定制。

武汉菲恩生物科技有限公司.

湖北省武汉市东湖新技术开发区高新二路 388 号武汉光谷国际生物医药企业加速器 1.2 期 C22 栋一层、二层(430206)

性能介绍

用途	用于体外定量检测血清，血浆，细胞培养上清或其它生物样品中的 GSH。		
适用种属	Universal	实验方法	比色法
检测范围	1.56-100umol/L	灵敏度	1.2umol/L
检测时长	20 分钟(不含样品准备时间)		
单孔样品最大用量	血清：10ul；血浆：10ul；细胞培养上清：100ul；细胞裂解液或组织裂解液: 100ul；其它液体样品：100ul		
储存条件	未启封试剂盒 2-8°C(严禁冻存)，有效期见盒面标签		

背景简介

CAS: 70-18-8

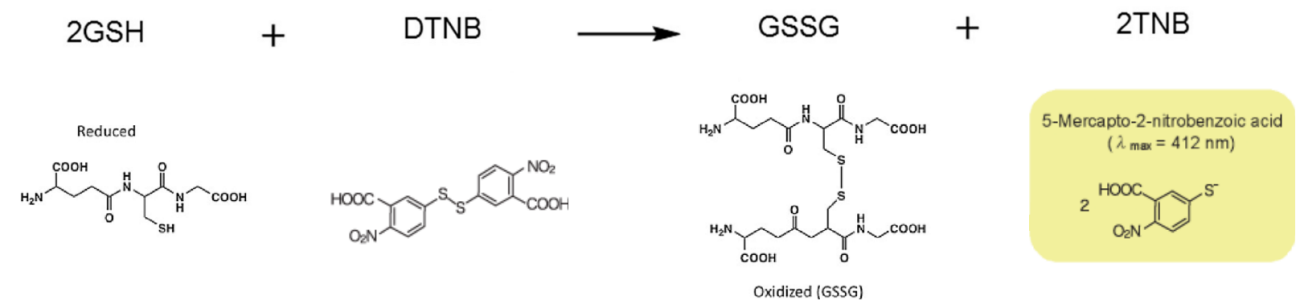
谷胱甘肽 (Glutathione, GSH)是一种由谷氨酸、半胱氨酸和甘氨酸组成的含有 γ-酰胺键和巯基的三肽，几乎存在于机体的每个细胞中。谷胱甘肽有助于维持正常的免疫系统功能，具有抗氧化和解毒作用。半胱氨酸上的巯基是其活性基团，容易与某些药物、毒素等结合，使其具有解毒作用。谷胱甘肽不仅可以用于医药，还可以作为功能食品的基础原料，广泛应用于延缓衰老、增强免疫力、抗肿瘤等功能食品中。
谷胱甘肽有两种形式:还原型谷胱甘肽 (G-SH)和氧化型谷胱甘肽 (G-S-S-G)，在生理条件下还原型谷胱甘肽占绝大多数。谷胱甘肽还原酶催化两种形式的相互转化，其辅酶也为磷酸戊糖旁路代谢提供 NADPH。定量 GSH 可以扩展到药物发现，药理学，毒理学研究和研究药物和有毒化合物对谷胱甘肽代谢的影响。

检测原理

谷胱甘肽(GSH)分光光度/酶标仪测定方法涉及 GSH 被 5,5 ' -二硫代-双(2-硝基苯甲酸)(DTNB)氧化形成黄色衍生物 5 ' -硫代-2-硝基苯甲酸(TNB)，在 412 nm(最大吸光值)测量。在 96 孔板中 GSH 的最低检出是 1.2umol。通过比较样品与标准曲线的 OD 值来确定样品中 GSH 的浓度。GSH 浓度与 OD 值成正比。

检测时间 20 分钟，包括试剂制备。试剂盒可以测定全血、血浆、血清、肺灌洗液、脑脊液、尿液、组织和细胞提取物中的 GSH。

反应机理：



各组件及开启后保存条件

未启封的试剂盒，请保存在 2-8°C。开启后，保存条件见如下表格所示：

组件	名称	规格(48T)	规格(96T)	保存条件
E001	ELISA Microplate 酶标板	8×6	8×12	保存在 2-8 度，6 个月
E002	GSH Standard（3.07mg） GSH 标准品粉末（3.07mg）	1vial	2vial	
E008	Standard stock solution 标准品储存液	1.5ml	1.5ml x 2	
E009	DTNB (Concentrated, 20X) DTNB（20x）	270ul	520ul	
E010	Assay Dilution Buffer 分析稀释液	11ml	22ml	
E007	Product Description 说明书	1 copy	1 copy	

注意：试剂瓶内提供的液体试剂体积比标签标明的稍多。请使用移液器精确量取并做相应比例稀释。

所需器材和试剂

1、 酶标仪(波长 405nm 或 414nm 滤光片)

2、 精密的单道(0.5-10μL, 5-50μL, 20-200μL, 200-1000μL)和多通道移液器(移液器使用前需校准)。

3、 无菌的 EP 管及一次性吸头

4、 10mM PBS PH7.2-8.0

样品采集及保存

1、血清

全血样本室温放置 2 小时或 2-8°C 过夜。1000×g 离心 20 分钟，取上清。可立即检测，或按一次使用量分装冻存于-20°C 或-80°C。

2、血浆

抗凝剂推荐使用 EDTA-Na2/K2，样品采集后 30 分钟内于 2-8°C，1000×g 离心 15 分钟，取上清。可立即检测，或按一次使用量分装冻存于-20°C 或-80°C。其他抗凝剂的使用及选择请查看样品制备指南。

3、组织样本

组织样本一般制成组织匀浆，处理方法如下：

3.1、将目标组织置于冰上，用预冷的 PBS 缓冲液(0.01M，pH=7.4)洗涤去除残留的血液，称重后备用。

3.2、在冰上用裂解液研磨组织匀浆。加入裂解液的体积取决于组织的重量，一般情况每 1g 组织碎片使用 9ml 裂解液。

3.3、可再利用超声破碎或反复冻融进一步处理(超声破碎过程中，需冰浴降温；反复冻融法可重复 2 次)。

3.4、将制备好的匀浆液于 5000×g 离心 5 分钟，留取上清即可检测。或按一次使用量分装冻存于-20°C 或-80°C。

3.5、根据实验需要，组织匀浆样本可先测定总蛋白浓度,以便于数据分析，推荐 BCA 法。一般调整总蛋白浓度至 1-3mg/ml 用于检测。

注意：裂解液常用 PBS 缓冲液，或使用 RIPA 裂解液。使用 RIPA 裂解液时， DTT 和还原剂组分会严重干扰试剂盒工作。

4、细胞培养上清

收集上清液，2-8°C，2500rpm 离心 5min，收集澄清的细胞培养上清。立即用于检测，或按一次使用量分装于-80°C 冻存备用。

5、细胞裂解液

5.1、悬浮细胞的收集及裂解：2-8°C，2500rpm 离心 5min，收集细胞。再加入预冷的 PBS 轻轻混匀清洗，2-8°C，2500rpm 离心 5min，收集细胞。置于冰上，裂解 30min-1h , 或者配合超声波破碎。

5.2、贴壁细胞的收集及裂解：吸走上清液，加入预冷的 PBS 洗三次。用细胞刮轻轻刮下贴壁细胞。细胞悬液转入离心管中，置于冰上，裂解 30min-1h，或者配合超声波破碎。

5.3、细胞裂解过程中可用枪头吹打或间断摇动离心管，使蛋白充分裂解, 出现黏糊状是 DNA，可以使用超声波破碎 DNA。(或用超声波 3-5mm 探头，功率 150-300W, 冰上超声处理样品，工作 1-2 秒，停止 30 秒， 3~5 个循环。)

5.4、裂解或超声破碎完成，2-8°C，10000rpm 离心 10min，上清移入 EP 管中，立即用于检测，或按一次使用量分装于-80°C 冻存备用。

注意：注意事项同组织样本。

6、其他生物样本

2-8°C，1000×g 离心样品 20 分钟。收集上清液立即用于检测，或按 一次使用量分装于-80°C 冻存备用。

样品其它注意事项

1、 收集血液的试管应为一次性无内毒素试管。避免使用溶血，高血脂样品。

2、 样品最佳保存条件：2-8°C 保存应小于 5 天，-20°C 不应超过 6 个月，-80°C 不应超过 2 年，超过以上时间应保存在液氮中。冻存的标本融化时，为了减少冰晶(0°C)对样品的破坏，应采用 15-25°C 水浴快速融化，融化后离心除去沉淀物，混匀后用于检测。

3、 试剂盒检测范围不等同于样本中待测物的浓度范围。如果样品中待测物浓度过高或过低，请对样本做适当的稀释或浓缩。

4、 若所检样本特殊，无参考数据，建议做预实验验证其有效性。

试剂盒使用注意事项

1、 试剂盒开启后，标准品的保存条件请参考组件保存条件表格(受潮后活性会下降)。如发生使用或保存不当导致组件缺损，可申请购买配套组件(如 E002 标准品)。

2、 请使用无菌一次性吸头吸取试剂，使用后，须旋紧试剂瓶盖，以防止微生物污染和蒸发。

3、 在未经确认的情况下，请勿将其他批次试剂盒的试剂或其他来源的试剂用于本试剂盒。

4、 请勿重复使用一次性吸头，以免造成交叉污染。

5、 试验中请穿实验服、戴口罩、手套等，做好防护工作。特别是检测血液或者其他体液样品时，请按国家生物试验室安全防护条例执行。

样品稀释方案推荐

以下表格为本试剂盒针对**有限样本**推荐的稀释比例，仅供参考。(ND 为未检出)

样本类型	推荐稀释比例	参考含量
Human serum (Healthy)	1/10-1/20	700umol/L
Human plasma (Healthy)	1/10-1/20	625umol/L
Rat serum (Healthy)	1/10-1/20	708umol/L
Mouse serum (Healthy)	1/10-1/20	620umol/L
T47D cell lysate	1/5-1/10	252umol/2mg (total protein)
Human urine (Healthy)	undiluted	7.2umol/L

如果您的模型组样本需要其他稀释比例，请参考如下通用稀释方案(此方案为检测不设置复孔的稀释方案。需要设置复孔时，请将样品及稀释液体积 x 复孔数)：

稀释 2 倍(1/2)：一步稀释。取 60ul 样品加入 60ul PBS 中，轻轻混匀。

稀释 5 倍(1/5)：一步稀释。取 24ul 样品加入 96ul PBS 中，轻轻混匀。

稀释 10 倍(1/10)：一步稀释。取 12ul 样品加入 108ul PBS 中，轻轻混匀。

稀释 20 倍(1/20)：一步稀释。取 6ul 样品加入 114ul PBS 中，轻轻混匀。

稀释 50 倍(1/50)：一步稀释。取 3ul 样品及 47ul PBS 加入 100ul PBS 中，轻轻混匀。

稀释 100 倍(1/100)：一步稀释。取 3ul 样品及 177ul PBS 加入 120ul PBS 中，轻轻混匀。

稀释 1000 倍(1/1000)：两步稀释，可先稀释 50 倍，再稀释 20 倍。轻轻混匀。

稀释 10000 倍(1/10000)：两步稀释，可先稀释 100 倍，再稀释 100 倍。轻轻混匀。

稀释 100000 倍(1/100000)：三步稀释，可先稀释 50 倍，再稀释 20 倍，最后稀释 100 倍。轻轻混匀。

注意：每步稀释时取液量不少于 3ul，稀释倍数不超过 100 倍。每步稀释都需混合均匀，避免起泡。

检测前试剂准备

从冰箱中取出试剂盒，无需平衡，可直接使用。如果试剂盒需分多次使用，请仅取出本次实验所需的酶标板孔，剩余试剂按指定条件保存。

1、10mM GSH 浓缩标准品的配制:

1.1 将标准品管 10000xg 离心 1min。将粉末离心至管底。标记为 10mM GSH。

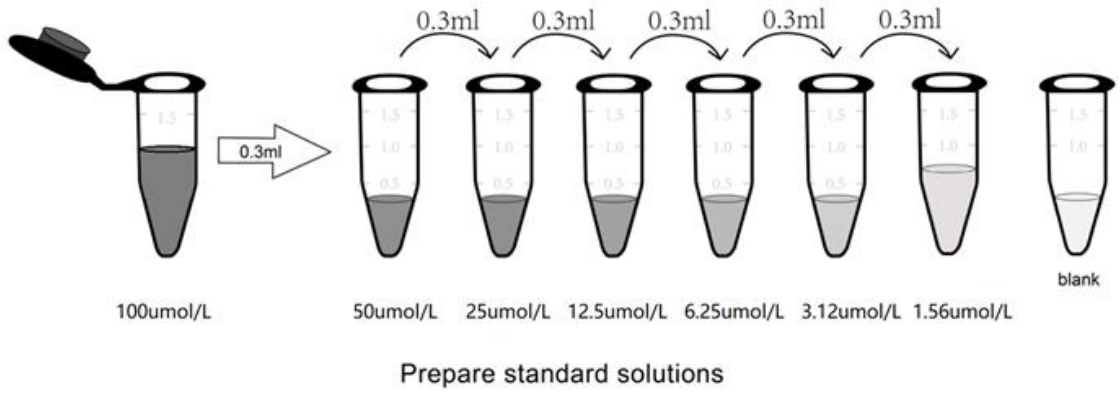
1.2 加入 1ml 标准品储存液。拧紧管盖，将管颠倒几次，使其轻轻混合。(也可以用低速涡旋仪混匀 20 秒。)

1.3 以 1000xg 离心 10 秒，将液体收集至管底。

2、标准品的稀释:

2.1、取一个干净的 EP 管，标记为 Zero tube。在 EP 管中加入 990ul 分析稀释液。再加入 10ul 10mM GSH 浓缩标准品。将 EP 管上下颠倒数次轻轻混匀。(也可以用低速涡旋仪混匀 3-5 秒。) 1000×g 低速离心 1 分钟，将液体收集至管底。

2.2、梯度稀释：另取 7 个 EP 管，分别标记为 1/2、1/4、1/8、1/16、1/32、1/64 和 blank。先在每个 EP 管中分别加入 0.3ml 的分析稀释液。再取 0.3ml Zero tube 标准品溶液加入到 1/2 管中，充分混合。再取 0.3ml 1/2 管标准品溶液到 1/4 管中，充分混合。再取 0.3ml 1/4 管标准品溶液到 1/8 管中，充分混合，依此类推。注意 Blank EP 管中仅有分析稀释液。此时，从 Zero tube 管到 blank 管这 8 个 EP 管中标准品的浓度分别为 100umol/L, 50umol/L, 25umol/L, 12.5umol/L, 6.25umol/L, 3.125umol/L, 1.562umol/L, 0umol/L。



Prepare standard solutions

注: 10mM GSH 浓缩标准品，-20℃可保存 6 个月，2-8℃可保存 2 个月。已稀释过的其他梯度标准品工作液请于 2 小时内使用。

3、DTNB 工作液的制备：

加样前 5 分钟内准备好，现用现配，不能长时间保存。

3.1、计算所需工作液的总体积：100ul/孔×孔数。(最好准备比总体积多 100ul-200ul 的量)

3.2、1000×g 低速离心 1 分钟，将浓缩 DTNB（20X）收集至管底。

3.3、用分析稀释液按 1/20 的比例稀释浓缩 DTNB，充分混匀。(如将 50ul 浓缩 DTNB 加入 950ul 分析稀释液中)

详细操作步骤

稀释样品和试剂时，需将它们完全混合。每次测试均需绘制标准曲线。

1. 在板上分别设置标准品、样品、对照(空白)孔，并记录其位置。建议对每个标准品和样品进行重复测量，以减少实验误差。

2. 向标准品孔中加入 100ul 各梯度标准品，向样品孔中加入 100ul 适度稀释的待测样品，向空白孔中加入 100ul 分析稀释液。

3. 加 DTNB 工作溶液:向每孔中添加 100ul DTNB 工作溶液。轻轻震荡酶标板混匀工作液，在室温下孵育 3 分钟。

4. OD 测量:立即用酶标仪在 405nm 或 414nm 处读取 OD 吸光度。

结果计算

1、 取标准品和样品复孔的平均 OD 值。

2、 以浓度为横坐标，平均 OD 值为纵坐标，可使用 Linear Fit 绘制标准曲线。也可使用酶标仪自带的作图软件(如 Thermo FC 型号酶标仪 SkanIt RE 软件)，或 Curve Expert 1.3 or 1.4 专业软件(本公司网站可以免费下载使用)绘制标准曲线。

3、 将样本的 OD 值代入标准曲线，即可计算得到样品的浓度值。 如果样品被稀释过，则需乘以相应的稀释倍数。

样品类型	举例方程: Linear Fit: y= a + bx (a=0.0819, b=0.0043)
血清，血浆，脑脊液，尿液	(样品的 GSH 浓度 umol/L) = (△A405 − a) ÷ b × f
裂解液: C =g/L (BCA 法测试)	(样品的 GSH 浓度 umol/g) = (△A405 − a) ÷ b × f ÷ C

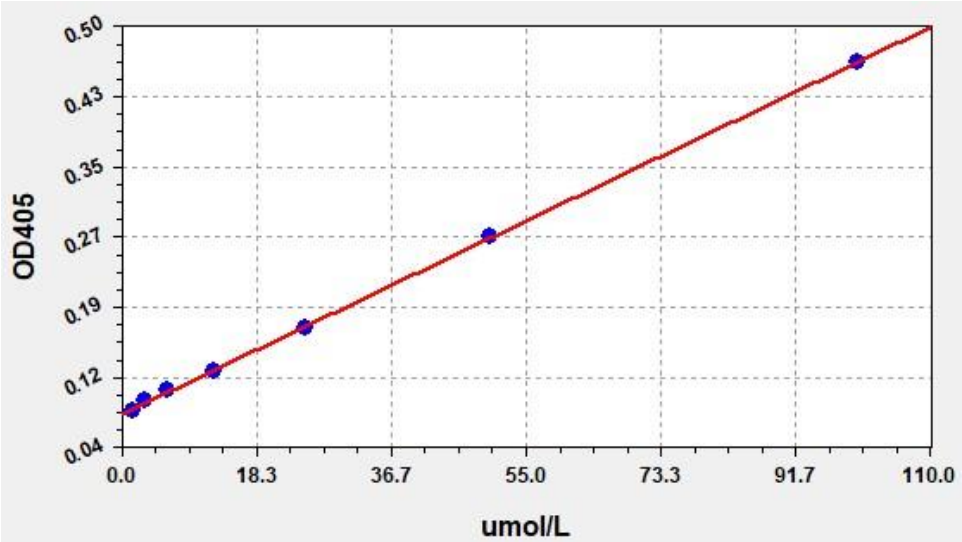
注:f(样品稀释倍数)，C(样品总蛋白浓度)

实验数据及标准曲线

本产品经品管部检测，符合使用手册的性能要求。(实验室湿度为 20%-60%，温度为 18°C -25°C。)

因具体实验环境及操作存在差异，以下实验数据和标准曲线仅供参考，实验人员需根据自己的实验建立标准曲线。（常用 OD414 或 OD405 来测量，此处选择 OD405。使用 Curve Expert 1.3 or 1.4 作图。）

STD.(umol/L)	OD-1	OD-2	Average
0	0.079	0.08	0.08
1.562	0.083	0.083	0.083
3.125	0.092	0.092	0.092
6.25	0.103	0.104	0.104
12.5	0.125	0.126	0.126
25	0.172	0.174	0.173
50	0.272	0.275	0.274
100	0.464	0.462	0.463



精密度

板内精密度：低、中、高浓度样本分别在同 1 块酶标板上检测 20 次。

板间精密度：低、中、高浓度样本分别在 3 块酶标板上检测 20 次。

类别	板内变异系数			板间变异系数		
样本	1	2	3	1	2	3
数量	20	20	20	20	20	20
平均值(umol/L)	3	13.36	50.16	3.04	13.9	51.47
标准差	0.13	0.57	2.35	0.16	0.77	3.25
变异系数(%)	4.23	4.23	4.68	5.22	5.54	6.32

回收率

将一定含量的 GSH 加入样本中，并通过将测量值与样品中 GSH 的预期量进行比较来计算回收率。

样品类型	回收率范围 (%)	平均回收率 (%)
血清(n=10)	88-100	95
EDTA 血浆(n=10)	88-103	97
肝素血浆(n=10)	86-102	95

线性

将添加有适当浓度 GSH 的样品分别稀释 2 倍、4 倍、8 倍来检测，得出回收率范围。

样品类型	1:2	1:4	1:8
血清((n=10)	91-103%	90-101%	85-99%
EDTA 血浆(n=10)	95-104%	87-99%	83-95%
肝素血浆(n=10)	99-105%	89-101%	84-99%

疑难解答提示

若实验结果不理想，请及时将显色结果拍照并保存实验数据，保留所用板条及未使用试剂。凭试剂盒盒面货号及批次号，联系我公司销售人员为您解决问题。同时您也可以参考以下表格自查原因：

问题描述	可能原因	相应对策
标准曲线无信号	标准液和 DTNB 工作液被错误稀释	确认各步骤所加试剂正确，可重做一次并确认
	GSH 标准品储存不当，或过期	重新制备 GSH 标准品
	漏加了试剂	确认试剂是否添加
标准曲线显色过强	混淆了不同试剂盒的组件，或配置工作液浓度过高	使用试剂盒本身的各组件，可重做一次并确认
样品无信号	待测样品含量低于测定的检测限	减小稀释倍数或浓缩样品
	目的物和缓冲液的相容性不好	确保样品储存缓冲液与待测样品相容性
	样品制备不正确	参考样品制备指南并规范保存
	样品保存时间过久	按一次使用量分装并规范保存
变异系数 (CV) 较大	样品 OD 值超过标准曲线最高浓度。	增大样品的稀释倍数
	酶标板不干净	实验时勿碰触酶标板底部
	孔中有气泡	确保酶标板读数时孔中无气泡
	试剂未混匀	所有试剂已充分混合
	移液量不一致	使用校准好的移液器和正确的移液方法
标准曲线信号弱	标准品配置不当	开盖前短暂离心冻干标准品管；检查是否溶解完全
	标准品已降解	按推荐方式保存标准品
	移液体积出错或不准	使用校准好的移液器和正确的移液方法
	试剂盒过期	不使用过期产品
	试剂盒保存不当	按说明书要求保存各组分
	酶标仪波长不正确	核实波长并重新读取 OD405 或 OD414
背景高	检测试剂过多，或配置浓度过高	使用校准好的移液器和正确的移液方法
	完成测试后读数不及时	测试后立即读数
	DTNB 工作液被污染	使用干净的 EP 管重新配置工作液

声明

1、 限于现有条件及科学技术水平，尚不能对所有原料进行全面的鉴定分析，本产品可能存在一定的质量技术风险。

2、 本试剂盒在研发过程中去除/降低了生物学样本中的一些内源性干扰因素，并非所有可能影响的因素均已去除。

3、 最终的实验结果与试剂的有效性、实验者的相关操作以及当时的实验环境等因素密切相关，本公司只对试剂盒本身负责，不对因使用试剂盒所造成的样本消耗负责，请使用者使用前充分考虑到样本可能的使用量，预留充足的样本。

4、 为了达到好的实验结果，请只使用本公司试剂盒内提供的试剂，不要混用其他制造商的产品，严格按照说明书操作。

5、 由于操作过程中试剂制备以及酶标仪参数设置不正确，可能导致结果异常，实验前请仔细阅读说明书并调整好仪器。

6、 即使是相同人员操作也可能在两次独立实验中得到不同的结果，为保证结果的重现性，需要控制实验过程中每一步的操作。

7、 试剂盒发货前会经过严格的质检，然而，因为运输条件、实验设备差异等等因素影响，用户检测结果可能跟出厂数据不一致。不同批次间试剂盒间的差异也可能来自上述原因。

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(For Research Use Only. Not For Use In Diagnostic Procedures!)

FineTest®

GSH (Reduced Glutathione) colorimetric Assay Kit

Catalogue No.: EU2640

Revision: V4.0

Size: 48T/96T

Please do not mix and use reagents from different kits or different batches. Otherwise, it might not work properly.

Please read the manual carefully before use. Feel free to contact us if you have any questions.

Tel	027-86697005
Email	sales2@fn-test.com
Website	https://www.fn-test.cn/

Please provide the batch number (see kit label) for more rapid response and services.

It's strongly recommended to use this kit within the expiry date printed on the kit label.

Wuhan Fine Biotech Co., Ltd

1-2 Floor, BLD 22, Optics Valley Biopharmaceutical Accelerator,

No.388 Gaoxin 2nd Road, East Lake High Tech Development Zone,

Wuhan, 430074, Hubei, China (430075)

Fax: (0086)027-87800889

Product Features

Application	In vitro quantitative determination of GSH concentrations in serum, plasma, cell culture supernatant and other biological samples.		
Reactivity	Universal	Detection Method	Colorimetric Assay
Range	1.56-100umol/L	Sensitivity	1.2umol/L
Detection Duration	20 mins (excluding sample preparation)		
Samples needed for single well(Max)	Serum: 10ul, Plasma: 10ul, Cell Culture Supernatant: 100ul, cell or tissue lysate: 100ul, Other liquid samples: 100ul		
Storage	2-8°C (for sealed box), please do not freeze! See kit label for expiry date		

Background

CAS: 70-18-8

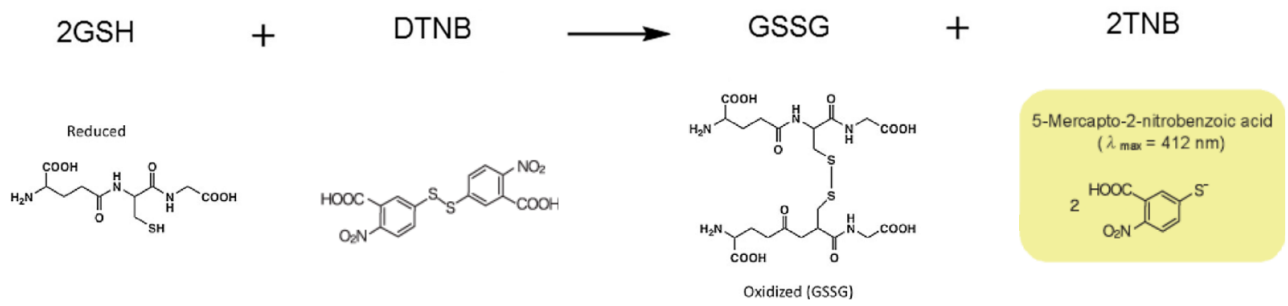
Glutathione (GSH) is a tripeptide containing γ-amide bond and sulfhydryl group, which is composed of glutamate, cysteine and glycine, and is present in almost every cell of the body.
Glutathione can help maintain normal immune system function, and has antioxidant effects and integrated detoxification. The sulfhydryl group on cysteine is its active group (used to be abbreviated as G-SH), which is easy to combine with some drugs, toxins, etc., so that it has an integrated detoxification effect. Glutathione can not only be used in medicine, but also as the base material of functional food, which is widely used in delaying aging, enhancing immunity, anti-tumor and other functional foods.
There are two forms of glutathione, reduced (G-SH) and oxidized (G-S-S-G), with reduced glutathione accounting for the vast majority under physiological conditions. Glutathione reductase catalyzes the interconversion between the two forms, and its coenzyme also provides NADPH for pentose phosphate bypass metabolism. Quantification of GSH can be extended to drug discovery, pharmacology, toxicology studies and to study the effects of drugs and toxic compounds on glutathione metabolism.

Principle of the Assay

The spectrophotometric/microplate reader assay method for glutathione (GSH) involves oxidation of GSH by the 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5′-thio-2-nitrobenzoic acid (TNB), measurable at 412 nm. The lowest detection for GSH is 1.2umol in a 96-well plate. The concentration of GSH in the samples is determined by comparing the OD of the samples to the standard curve. The concentration of GSH is proportional to the OD value.

The kit is rapid and the whole procedure takes no longer than 20 min including reagent preparation. It can assay GSH in whole blood, plasma, serum, lung lavage fluid, cerebrospinal fluid, urine, tissues and cell.

Reaction mechanism：



Kit Components and Storage

The sealed kit can be stored at 2-8 ℃. The storage condition for opened kit is specified in the table below:

No.	Item	Size(48T)	Size(96T)	**Storage Condition**
E001	ELISA Microplate	8×6	8×12	Stored for 6 months at 2-8°C
E002	GSH Standard（3.07mg）	1vial	2vial	
E008	Standard stock solution	1.5ml	1.5mlx2	
E009	DTNB (Concentrated, 20X)	270ul	520ul	
E010	Assay Dilution Buffer	11ml	22ml	
E007	Product Description	1 copy	1 copy	

Note: The liquid reagent bottle contains slightly more reagent than indicated on the label. Please use pipette accurately measure and do proportional dilution.

Required Instruments and Reagents

1. Microplate reader (wavelength: 405 or 414nm)
2. Precision single (0.5-10μL, 5-50μL, 20-200μL, 200-1000μL) and multi-channel pipette with disposable tips(calibration is required before use.)
3. Sterile tubes and Eppendorf tubes with disposable tips
4. 10mM PBS PH7.2-8.0

Sample Collection and Storage

1. Serum

Place whole blood sample at room temperature for 2 hours or at 2-8°C overnight. Centrifuge for 20min at 1000xg and collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

2. Plasma

EDTA-Na_2/K_2 is recommended as the anticoagulant. Centrifuge samples for 15 minutes at 1000×g 2-8°C within 30 minutes after collection. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay. For other anticoagulant types and uses, please refer to the sample preparation guideline.

3. Tissue Sample

Tissue samples are required to be made into homogenization. Protocol is as below:

3.1. Place the target tissue on the ice. Remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Then weigh for usage.

3.2. Use lysate to grind tissue homogenates on the ice. The adding volume of lysate depends on the weight of the tissue. Usually, 9mL PBS would be appropriate to 1 gram tissue pieces.

3.3. Do further process using ultrasonic disruption or freeze-thaw cycles (Ice bath for cooling is required during ultrasonic disruption; Freeze-thaw cycles can be repeated twice.) to get the homogenates.

3.4. Homogenates are then centrifuged for 5 minutes at 5000×g. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

3.5. Determine total protein concentration by BCA kit for further data analysis. Usually, total protein concentration for assay should be within 1-3mg/ml.

Notes: PBS buffer or the RIPA lysis can be used as lysates. Avoid using any reagents containing DTT Or reducing agent due to their severe interfere with kits' working.

4. Cell Culture Supernatant

Collect the supernatant: Centrifuge at 2500 rpm at 2-8℃ for 5 minutes, then collect clarified cell culture supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

5. Cell Lysate

5.1. Suspension Cell Lysate: Centrifuge at 2500 rpm at 2-8℃ for 5 minutes and collect cells. Then add pre-cooling PBS into collected cell and mix gently. Recollect cell by repeating centrifugation. Add 0.5-1ml cell lysate. Lyse the cell on ice for 30min-1h or disrupt the cell by ultrasonic disruption.

5.2. Adherent Cell Lysate: Absorb supernatant and add pre-cooling PBS to wash three times. Add 0.5-1ml cell lysate. Scrape the adherent cell with cell scraper. Lyse the cell suspension added in the centrifuge tube on ice for 30min-1h or disrupt the cell by ultrasonic disruption.

5.3. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Mucilaginous product is DNA which can be disrupted by ultrasonic cell disruptor on ice. (3~5mm probe, 150-300W, 3~5 s/time, 30s intervals for 1~2s working).

5.4. At the end of lysate or ultrasonic disruption, centrifuge at 10000rpm at 2-8℃ for 10 minutes. Then, the supernatant is added into EP tube to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

Notes: Read notes in tissue sample.

6. Other Biological Sample

Centrifuge samples for 15 minutes at 1000×g at 2-8℃. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

Notes for Samples

1. Blood collection tubes should be disposable and non-endotoxin. Avoid to use hemolyzed and lipemia samples.

2. The best sample storage condition: less than 5 days at 2-8℃; within 6 months at -20℃; within 2 years at -80℃. Stored in liquid nitrogen for a longer storage. When melting frozen samples, rapid water bath at 15-25℃ can decrease the effect of ice crystal (0℃) on the sample. After melting, centrifuge to remove the precipitate, and then mix well.

3. The detection range of this kit is not equivalent to the concentration of analyze in the sample. For analyses with higher or lower concentration, please properly dilute or concentrate the sample.

4. Pretest is recommended for special samples without reference data to validate the validity.

Precautions for Kits

1. After opening the kit, please refer to the table of storage condition for GSH standards (Dampness may decrease the activity.). If any component is missing or damaged during the assay or storage, please contact us for ordering a new one to replace. (e.g. E002 GSH standard)

2. Sterile and disposable tips are required during the assay. After use, the reagents bottle cap has to be tightened to avoid the microbial contamination and evaporation.

3. Before confirmation, reagents from other batches or sources should not be used in this kit.

4. Don't reuse tips and tubes to avoid cross contamination.

5. Please wear the lab coat, mask and gloves to protect yourself, during the assay. Especially, for the detection of blood or other body fluid sample, please follow regulations on safety protection of biological laboratory.

Recommended Sample Dilution Ratio

Random individual test data:

Sample Type	Recommended Dilution Ratio	Content
Human serum (Healthy)	1/10-1/20	700umol/L
Human plasma (Healthy)	1/10-1/20	625umol/L
Rat serum (Healthy)	1/10-1/20	708umol/L
Mouse serum (Healthy)	1/10-1/20	620umol/L
T47D cell lysate	1/5-1/10	252umol/2mg (total protein)
Human urine (Healthy)	undiluted	7.2umol/L

If other dilution ratio for your sample model is required, please refer to the universal dilution ratio below. (The ratio is suitable for single-well assay. For duplicate assay, please follow the calculation: volume of sample and diluent x number of duplicate well)

For 2 fold dilution (1/2): One step dilution. Add 60µL sample into 60µL PBS and mix gently.

For 5 fold dilution (1/5): One step dilution. Add 24µL sample into 96µL PBS and mix gently.

For 10 fold dilution (1/10): One step dilution. Add 12µL sample into 108µL PBS and mix gently.

For 20 fold dilution (1/20): One step dilution. Add 6µL sample into 114µL PBS and mix gently.

For 50 fold dilution (1/50): One step dilution. Add 3µL sample and 47µL PBS into 100 µL PBS and mix gently.

For 100 fold dilution (1/100): One step dilution. Add 3µL sample and 177µL PBS into 120µL PBS and mix gently.

For 1000 fold dilution (1/1000): Two step dilution. Create a 50-fold dilution first. Then, create a 20-fold dilution and mix gently.

For 10000 fold dilution (1/10000): Two step dilution. Create a 100-fold dilution first. Then, create the same dilution again and mix gently.

For 100000 fold dilution (1/100000): Three step dilution. Create a 50-fold dilution and 20-fold dilution respectively .Finally, create a 100-fold dilution and mix gently.

Notes: The volume in each dilution is not less than 3µL. Dilution factor should be within 100 fold. Mixing during dilution is required to avoid foaming.

Reagent Preparation and Storage

The kit is ready for immediate use when taken out of the refrigerator.

1. 10mM GSH standard storage solution

1.1 Centrifuge GSH standards tube for 1min at 10000xg. Label it as 10mM GSH.

1.2 Add 1ml Standard stock solution buffer into the standard tube. Tighten the tube cap and invert the tube several times to mix gently. (Or you can mix it using a low speed vortex mixer for 20 seconds.)

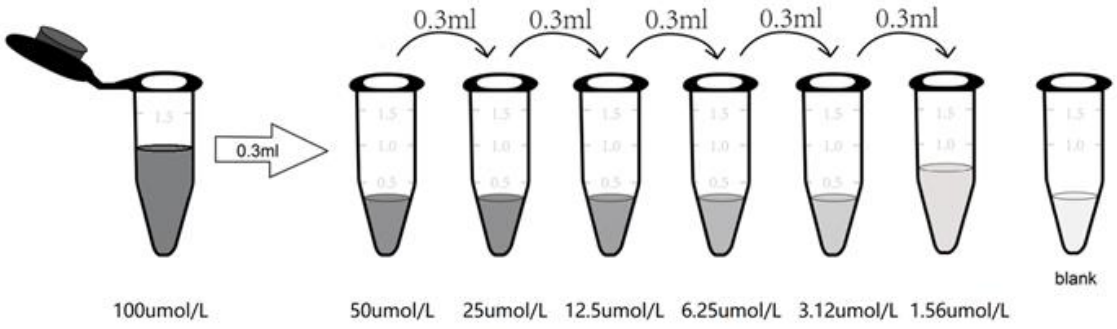
Centrifuge the tubes for 10s at 1000xg, making the liquid towards the bottom of tube.

2. Standards

2.1. Label an EP tube with zero tube. Add 990ul Assay Dilution Buffer and 10ul **10mM GSH standard storage solution** into it. Invert the tube several times to mix gently. (Or you can mix it using a low speed vortex mixer for 3-5 seconds.)

2.2. Centrifuge the tubes for 1min at 1000xg, making the liquid towards the bottom of tube and removing possible bubbles.

2.3. Standard dilution: Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the Assay Dilution Buffer into each tube. Add 0.3ml solution from zero tube into 1/2 tube and mix them thoroughly. Transfer 0.3ml from 1/2 tube into 1/4 tube and mix them thoroughly. Transfer 0.3ml from 1/4 tube into 1/8 tube and mix them thoroughly, so on till 1/64 tube. Now blank tube only contain 0.3ml Assay Dilution Buffer. The standard concentration from zero tube to blank tube is 100umol/L, 50umol/L, 25umol/L, 12.5umol/L, 6.25umol/L, 3.125umol/L, 1.562umol/L, 0umol/L.



Prepare standard solutions

Notes: The 10mM GSH standard storage solution can be stored at -20 ° C for 6 months or at 2-8 ° C for 2 months. Other diluted working solutions containing standards should be used in 2h.

3. Preparation of DTNB Working Solution

The working solution should be prepared within 5min before the assay and can't be stored for a long time.

3.1. Calculate required total volume of the working solution: 100ul/well x quantity of wells. (It's better to prepare additional 100ul-200ul.)

3.2. Centrifuge for 10s at 1000xg in low speed and bring down the concentrated DTNB (20X) to the bottom of tube.

3.3. Dilute the DTNB with Assay Dilution Buffer at 1/20 and mix them thoroughly. (e.g. Add 50ul concentrated DTNB into 950ul Assay Dilution Buffer.)

Detailed Assay Procedure

When diluting samples and reagents, they must be mixed completely. It's recommended to plot a standard

1. Set standard, pilot samples, control (blank) wells on the plate respectively, and then, records their positions. It's recommended to measure each standard and sample in duplicate to decrease experimental errors.

2. Standards and samples loading: Aliquot 100ul of zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube into each standard well. Also add Assay Dilution Buffer into the control (blank) well. Then, add 100ul pilot samples into each sample well.

3. DTNB Working Solution loading: Add 100ul DTNB Working Solution into each well. Then gently shake the plate to mix the working solution and incubated for 3 minutes at room temperature.

4. OD Measurement: Read the O.D. absorbance at 405nm or 414nm in a microplate reader immediately.

Calculation of Results

1. Calculate the mean OD value of the duplicate readings for each standard, control, and sample.

2. Create a Linear Fit curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. Alternatively, you can use the curve fitting software offered by the microplate reader (e.g. Thermo SkanIt RE software, Curve Expert 1.3 or 1.4 available in FineTest website).

3. Calculate the sample concentration by substituting OD value into the standard curve. Diluted samples should be multiplied by the relevant dilution ratio.

sample type	IE: Linear Fit: y= a + bx (a=0.0819, b=0.0043)
Serum, plasma, CSF, urine	(GSH of sample umol/L) = (△A405 − a) ÷ b × f
Lysate: C=g/L (BCA method)	(GSH of sample umol/g) = (△A405 − a) ÷ b × f ÷ C

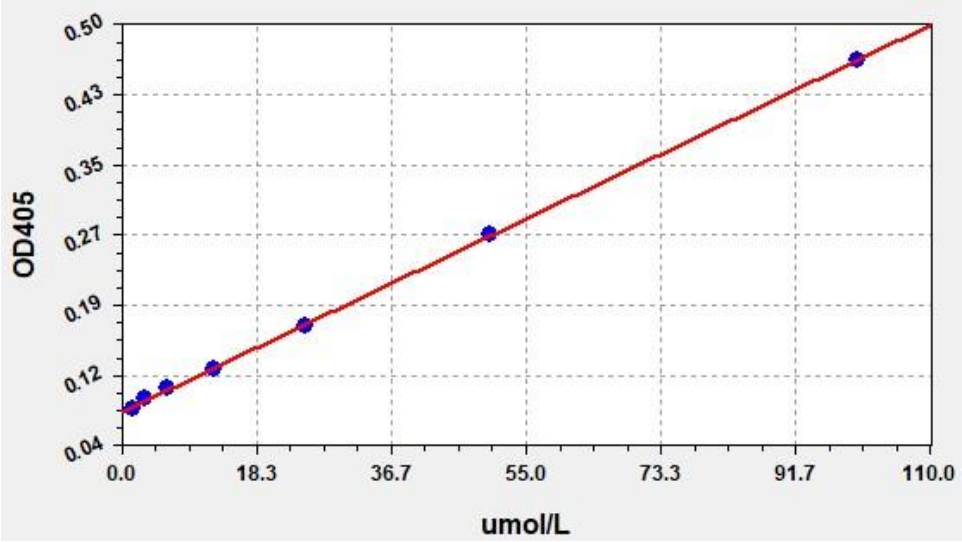
Note： f (sample dilution factor), C (total protein concentration of the samples)

Typical Data & Standard Curve

This product has been tested by Quality Control Department and meets performance specifications mentioned in the manual. (The humidity in the laboratory is 20%-60%, and the temperature is 18°C -25°C.)

The following assay data are provided for reference, since experimental environment and operation are different. The establishment of standard curve depends on your own assay.

STD.(umol/L)	OD-1	OD-2	Average
0	0.079	0.08	0.08
1.562	0.083	0.083	0.083
3.125	0.092	0.092	0.092
6.25	0.103	0.104	0.104
12.5	0.125	0.126	0.126
25	0.172	0.174	0.173
50	0.272	0.275	0.274
100	0.464	0.462	0.463



Precision

Intra-assay Precision: samples with low, medium and high concentration are tested 20 times on same plate.

Inter-assay Precision: samples with low, medium and high concentration are tested 20 times on three different plates.

Item	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (umol/L)	3	13.36	50.16	3.04	13.9	51.47
Standard deviation	0.13	0.57	2.35	0.16	0.77	3.25
CV(%)	4.23	4.23	4.68	5.22	5.54	6.32

Recovery

Add a certain amount of GSH into the sample. Calculate the recovery by comparing the measured value with the expected amount of GSH in the sample.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	88-100	95
EDTA Plasma(n=5)	88-103	97
Heparin Plasma(n=5)	86-102	95

Linearity

Dilute the sample with a certain amount of GSH at 1:2, 1:4 and 1:8 to get the recovery range.

Sample	1:2	1:4	1:8
Serum(n=5)	91-103%	90-101%	85-99%
EDTA Plasma(n=5)	95-104%	87-99%	83-95%
Heparin Plasma(n=5)	99-105%	89-101%	84-99%

Troubleshooting

If the result is unsatisfied, please take a screenshot for the staining result and store the OD data. Keep used strips as well the rest reagents. Contact us to solve your problem with the kit's catalogue number and batch number. You can also refer to the following table to check the reason.

Problem	Possible Causes	Solutions
Standard curve without signal	The standard and DTNB working solution were incorrectly diluted	Confirm the required reagent added in each step. Also repeat the assay and verify.
	GSH standard is stored improperly, or expired	The GSH standards were re-prepared
	Forget to add some reagents	Verify whether the required reagent is added.
Overflow OD	Use components from different kits, or prepare the working solution with higher concentration	Use the component included in the same kit. Also repeat the assay and verify.
Samples without signal	The amount of pilot sample is lower than the detection range.	Decrease dilution ratio or concentrate the sample.
	The detection target is incompatible with the buffer.	Verify the compatibility of sample storage buffer with the pilot sample.
	Incorrect preparation of sample	Please refer to sample preparation guideline and regularly store.
	Longer storage of sample	Aliquot and store samples according to the assay requirement.
High CV%	The OD value of the sample exceeded the highest concentration of the standard curve.	Increase the dilution ratio of the sample.
	Unclean plate	Don't touch the bottom of the plate during the assay.
	Foam is found in the well.	Avoid foaming during reading in a microplate reader.
	Reagents are not completely mixed.	Mix all reagents completely.
	Inconsistent pipetting	Use calibrated pipette and correct pipetting method.
Standard curve with low signal	Standards are improperly reconstituted.	Before opening, shortly centrifuge the standard tube till complete dissolution.
	Standards have been degraded.	Follow suggested storage conditions for standards.
	When pipetting, the required volume is incorrect or inaccurate.	Use calibrated pipette and correct pipetting method.
	Expired kit	Don't use expired products.

	Improper storage	Follow suggested storage conditions for all components.
	The wavelength of the microplate reader is incorrect.	Check the wavelength and read the OD405 or OD414 value again.
High Background	Too many detection reagents or higher concentration.	Use calibrated pipette and correct pipetting method.
	Reading of assay result is not in time.	Read the assay result immediately after test
	The DTNB working solution is contaminated	Reconfigure the working fluid using a clean tube

Declaration

1. Limited to current conditions and scientific techniques, all raw materials are not completely identified and analyzed. This product may have a technology-related quality risk.

2. During the Elisa kit development, some endogenous interferons(not all) in the biological sample have been removed or decreased.

3. The final assay result is related to the validity of reagents, experimental operation and environment. FineTest is only responsible for this kit, excluding sample consumption during using this kit. Before use, please consider and prepare enough samples required by the assay.

4. To get a satisfied assay result, please use all reagents offered by this kit. Don't use any product from other vendors. Strictly follow instructions of this manual.

5. During assay procedure, incorrect reagents preparation and parameter setting of the microplate reader may result in the abnormal result. Before assay, please read this manual carefully and adjust instruments.

6. Even if the assay is performed by the same person, results in two independent assays may be different. Thus, each step in the assay should be controlled to ensure the reproducibility.

7. Before delivery, this kit is subject to the strict QC. Influenced by transportation conditions and experimental devices, the assay result got by the customer may be different from original data. Inter-assay CV between different batches may be caused by reasons before.

8. This kit is not compared to similar kits from other vendors or methods for testing the same detection target. Thus, assay results may be inconsistent.

9. This kit allows for research use only. For IVD or other purposes, FineTest is not responsible for relevant consequences and doesn't bear any legal liability.