本试剂盒仅供体外研究使用，不用于临床诊断！

FineTest®

# 血红素（Heme）Assay 试剂盒

# Heme assay kit

产品货号：EU2610

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)

## 技术支持相关文件

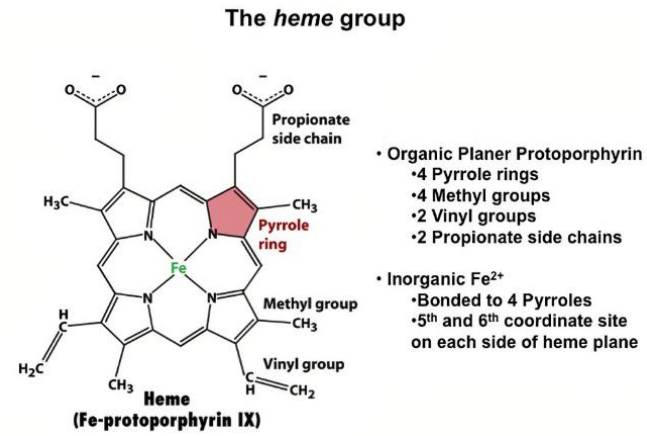
| 文件名称 | ELISA 实验手工洗板 | TMB 显色精准控制 | 标曲和浓度计算软件 CurveExpert1.4(含使用教程) |
|---|---|---|---|
| 网址 | https://www.fn-test.cn/videos/elisa-experiment-how-to-hand-wash-plate-operation-video/ | http://www.fn-test.cn/knowledge-share/tmb-color-rendering-precise-control/ | https://www.fn-test.cn/knowledge-share/elisa-standard-curve-draw/ |
| 二维码 | | | |

## 性能介绍

| 用途 | 用于体外定量检测（黄疸 溶血）血清血浆，红细胞裂解液或者含有 heme 的样品。 | | |
|---|---|---|---|
| 适用种属 | Universal | 实验方法 | 酶催化法 |
| 检测范围 | 15.6-1000pmol/ml | 灵敏度 | 5pmol/ml |
| 检测时长 | 20 分钟(不含平衡和样品准备时间) | | |
| 单孔样品最大用量 | 血清：3ul；血浆：3ul；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：50ul | | |
| 特异性 | 特异性和 Heme 结合，与其它类似物无明显交叉反应 | | |
| 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

血红素是血红蛋白的一种非蛋白质铁卟啉成分，也存在于其他呼吸色素、动物/植物细胞中。血红素是原卟啉的亚铁衍生物，是一种由 4 个吡咯环与铁原子相连的配位螯合物。血红素是血红蛋白和一些氧化还原酶的辅基，参与机体的氧转移和氧化还原。脂溶性非偶联胆红素在肝细胞内经过一系列酶的处理，转化为水溶性偶联胆红素 (直接胆红素)。由于红细胞受损、肝负荷增加、肝细胞运输、结合和排泄障碍或肝外胆道梗阻，血液中胆红素浓度升高可导致黄疸。



## 检测原理

该试剂盒基于酶检测法,活化的 heme 可以将 TMB 氧化为蓝色溶液（最大吸收峰 630nm）。试剂盒中提供 heme 标准品和活化稀释液。依次将活化标准品、稀释的样品加入孔中，再将 TMB 底物溶液添加到每个孔中进行显色（蓝色）。通过添加硫酸溶液终止酶-底物反应，并通过分光光度法在 450nm 的波长处测量 OD 值。使用曲线方程软件，将样品的 OD450 值与标准曲线进行比对，确定样品中待测 heme 的浓度。

## 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。

| Item | Specifications(48T/96T) | Storage |
|---|---|---|
| ELISA Microplate（反应微孔板） | 8×6/8×12 | RT |
| 10×Heme Standard(liquid) 10nmol/ml（10 倍血红素标准品） | 60ul/120ul | 2-8°C |
| Activation diluent buffer（活化稀释液） | 10ml/20ml | 2-8°C |
| TMB Substrate（显色底物） | 5ml/10ml | 2-8°C(Avoid Direct Light) |
| Stop Solution（终止液） | 5ml/10ml | 2-8°C |
| Plate Sealer（封板贴） | 3/5pieces | |
| Product Description（说明书） | 1copy | |

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。

## 所需器材和试剂

1、 酶标仪(波长 450nm 滤光片)

2、 37°C 恒温箱(不推荐使用细胞用 $CO_2$ 培养箱)

3、 自动洗板机或多道移液器/5ml 滴管(手工洗板用)

4、 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)。

5、 无菌的 EP 管及一次性吸头

6、 吸水纸及加样槽

7、 去离子水或蒸馏水

## 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，用预冷的生理盐水(即 0.9%氯化钠)洗涤去除残留的血液，称重后备用。

3.2、在冰上用生理盐水研磨组织匀浆。加入生理盐水的体积取决于组织的重量，一般情况每 1g 组织碎片使用 9ml 生理盐水。另外建议在生理盐水中加入蛋白酶抑制剂，如 1mM PMSF。

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 ELISA 检测。

**注意：不建议使用其他裂解液，会导致 TMB 孵育时沉淀和不显色。**

### 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。再加入预冷的生理盐水(即 0.9%氯化钠)轻轻混匀清洗，2-8°C，2500rpm 离心 5min，收集细胞。加入 0.5-1ml 生理盐水(即 0.9%氯化钠)及适量蛋白酶抑制剂(如 PMSF，工作浓度 1mmol/L)，置于冰上，配合超声波破碎。

5.2、贴壁细胞的收集及裂解：吸走上清液，加入预冷的生理盐水洗三次。加入 0.5-1ml 生理盐水及适量蛋白酶抑制剂(如 PMSF，工作浓度 1mmol/L)，用细胞刮轻轻刮下贴壁细胞。细胞悬液转入离心管中，配合超声波破碎(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。)。

5.3、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。

**样本处理相关试剂推荐：**100mM PMSF 蛋白酶抑制剂，货号 E051。

## 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。

5、 重组蛋白可能与试剂盒中捕获或检测抗体不匹配而出现不能检测的情况。

## 试剂盒使用注意事项

1、 使用不同的试剂盒时，需先做好标记，防止组分混用，导致实验失败。

2、 试剂盒开启后，酶标板和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 冻干标准品)。

3、 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。

4、 手工洗板时，加洗液的吸头或滴管，切勿接触酶标板孔。不充分的洗涤或污染容易造成假阳性和高背景。

5、 检测过程中，请提前准备好下一步实验所需试剂，洗板后及时将试剂加入板孔，防止板孔干燥，导致检测失效。

6、 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。

7、 请勿重复使用一次性吸头，以免造成交叉污染。

8、 加样完成，贴覆膜以防孵育过程样品的蒸发，在推荐温度下完成孵育过程。

9、 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 plasma (n=5) | 1/100-1/200 | 9-13 nmol/ml (umol/L) |
| Human serum (n=5) | 1/100-1/200 | 6-21 nmol/ml (umol/L) |
| Rat serum (n=5) | 1/100-1/400 | 20-63 nmol/ml (umol/L) |
| mouse serum (n=5) | 1/100-1/400 | 24-77 nmol/ml (umol/L) |
| mouse whole blood (n=5) | 1/10000-1/400000 | 10 (mmol/L) |

**血清/血浆中的基质成分会影响检测结果，需用样品稀释液至少稀释 2 倍(1/2）！**

**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 样品稀释液中，轻轻混匀。

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

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

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

稀释 50 倍(1/50)：一步稀释。取 3ul 样品及 47ul 生理盐水(即 0.9%氯化钠)加入 100ul 样品稀释液中，轻轻混匀。

稀释 100 倍(1/100)：一步稀释。取 3ul 样品及 177ul 生理盐水加入 120ul 样品稀释液中，轻轻混匀。

稀释 1000 倍(1/1000)：两步稀释，可先稀释 50 倍(此步骤全使用生理盐水稀释)，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(此步骤全使用生理盐水稀释)，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(前两步全使用生理盐水稀释)，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。**

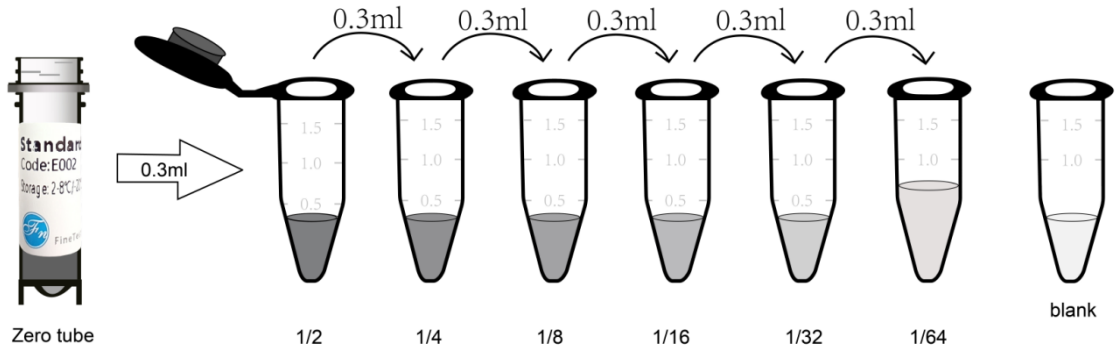
## 检测前试剂准备

提前 20 分钟从冰箱中取出试剂盒，平衡到室温(18-25°C)。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条及标准品，剩余酶标板条和标准品需按指定条件保存。

### 1、标准品:

1.1、将 450ul 活化稀释液加到空白 EP 管（标记为零号管-最高标准品浓度管），添加 50ul(10X 的 heme 标准品)然后充分混合（配置后为 1x 标准品工作液 1000pmol/ml）

1.2、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 的样品稀释液。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 管中仅有样品稀释液。此时，从 Zero tube 管到 blank 管这 8 个 EP 管中标准品的浓度分别为 1000pmol/ml, 500pmol/ml, 250pmol/ml, 125pmol/ml, 62.5pmol/ml, 31.25pmol/ml, 15.625pmol/ml, 0pmol/ml。




Prepare standard solutions

**注: 已溶解的零号管标准品，请保存于 2-8°C，并在 12 小时内使用。已稀释过的其他梯度标准品工作液请于 2 小时内使用。**

## 操作步骤概要

1、 设定标准品孔、待测样品孔、空白孔，并记录其位置。为减小实验误差，建议每个标准品和样品一式两份进行测量。

2、 **加样：**向相应孔中加入 100ul 标准品或待测样品。

3、 **加 TMB 底物溶液:** 每孔加入 100ul TMB 底物溶液，覆膜，在 37°C 下于暗箱中孵育 20 分钟。

4、 **加终止液:** 向每孔中添加 50ul 终止液。颜色将由蓝色立即变为黄色。添加终止液的操作顺序与添加 TMB 底物溶液的操作顺序相同。

5、 **OD 值的测量：**立即用酶标仪在 450nm 处读取 OD450 数值。（如果您的酶标仪有可以选择的校正波长，则设置为 570nm 或 630nm。校正读数值为 OD450 的值减去 OD570 或 OD630 的值。这种方式可以校正并去除非显色物质的 OD 值，从而获得更准确的检测结果。如果酶标仪没有 570nm 或 630nm 波长，则可使用原始 OD450 值。）

## 结果计算

### （操作视频：https://www.fn-test.cn/videos/standard-curve-drawing-video-in-elisa-kit/）

1、 取标准品和样品复孔的平均 OD450 值（使用原始 OD450 值或校正读数值），再减去空白孔的 OD450 值，作为计算值。

2、 以浓度为横坐标，OD450 值为纵坐标，可使用四参数方程 4PL 绘制标准曲线(作图时去掉空白孔的值)。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 OD450 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。

## 不同方法绘制标准曲线的说明

1、 线性图：一个坐标轴表示抗原的浓度，另一个表示读数 OD450 值。$R^2$ 值在此通常用于确定拟合，数值大于 0.99 表示拟合非常好。然而，线性图往往会压缩曲线下端上的数据点，导致计算结果不准确。

2、 半对数图：帮助抵消线性图引起的下端压缩。半对数图使用浓度的对数与读数的关系。这种方法通常会得到数据点分布更均匀的 s 形曲线。

3、 对数/双对数图：为低到中浓度范围提供良好的线性。但范围的高端则容易失去线性。

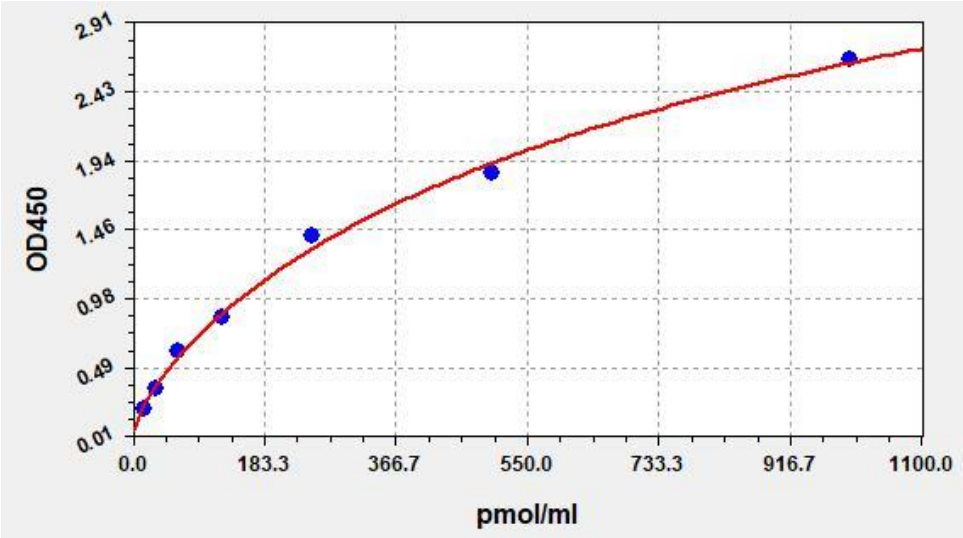
4、 四或五参数方程(4PL 或 5PL)曲线：方法更复杂，考虑了其他参数，比如最大值和最小值，因此需要更复杂的计算。4PL 假设拐点周围对称，而 5PL 考虑了不对称的情况，通常更适合免疫分析。如果您的软件允许，则 4-PL 和 5-PL 将适用于大部分 ELISA 校正标准曲线。

## 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。显色前将 TMB 平衡至 37°C，加入酶标板孔后，37°C 避光孵育 15 分钟。)

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。

| STD.(pmol/ml) | OD-1 | OD-2 | Average | Corrected |
|---|---|---|---|---|
| 0 | 0.069 | 0.071 | 0.07 | 0 |
| 15.625 | 0.206 | 0.212 | 0.209 | 0.139 |
| 31.25 | 0.341 | 0.351 | 0.346 | 0.276 |
| 62.5 | 0.597 | 0.615 | 0.606 | 0.536 |
| 125 | 0.834 | 0.858 | 0.846 | 0.776 |
| 250 | 1.401 | 1.441 | 1.421 | 1.351 |
| 500 | 1.829 | 1.883 | 1.856 | 1.786 |
| 1000 | 2.69 | 2.62 | 2.655 | 2.585 |



## 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(pmol/ml) | 30.53 | 120.9 | 508 | 31.06 | 124.3 | 503 |
| 标准差 | 1.49 | 5.42 | 24.38 | 1.36 | 5.84 | 21.83 |
| 变异系数(%) | 4.87 | 4.48 | 4.8 | 4.38 | 4.7 | 4.34 |

## 回收率

将一定含量的 Heme 加入样本中，并通过将测量值与样品中 Heme 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) | 88-98 | 94 |
| EDTA 血浆(n=10) | 89-100 | 95 |
| 肝素血浆(n=10) | 95-105 | 99 |

## 线性

将添加有适当浓度 Heme 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) | 87-105% | 86-101% | 84-100% |
| EDTA 血浆(n=10) | 85-94% | 86-95% | 80-94% |
| 肝素血浆(n=10) | 90-103% | 83-98% | 81-100% |

## 稳定性

未拆封的试剂盒在 37°C 和 2-8°C 进行稳定性实验，得出稳定性数据。

| 试剂盒(n=5) | 37°C 一个月 | 2-8°C 六个月 |
|---|---|---|
| 平均值 (%) | 80 | 95-100 |

## ELISA 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 | 检测试剂加样顺序不对 | 确认各步骤所加试剂正确，可重做一次并确认 |
| | 混淆了不同试剂盒的组件 | 使用试剂盒本身的各组件，可重做一次并确认 |
| | 漏加试剂 | 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 |
| 标准曲线图形不好 | 曲线选择不恰当 | 尝试使用不同方法绘制曲线 |
| 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 |
| | 样品保存时间过久或反复冻融 | 按一次使用量分装并规范保存 |
| 变异系数 (CV) 较大 | 显色时在孔中形成沉淀 | 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 |
| | 板孔洗涤不均 | 检查洗板机的管口是否畅通 |
| | 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 | 标准品复溶不当 | 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 |
| | 板孔过分干燥 | 检测及加样过程不可中断。尤其洗板之后，需及时加入试剂。孵育时，贴覆膜。 |
| | 酶反应显色慢 | 每次使用前将整瓶 TMB 显色底物在 37°C 平衡 30min。延长孵育时间 |
| | 酶标仪波长不正确 | 核实波长并重新读取 OD450 值 |
| | 板孔清洗过度 | 按说明书描述的洗板次数 |
| 背景高 | 未彻底洗涤 | 按说明书描述的洗板次数 |

| | | |
|---|---|---|
| | 洗涤缓冲液受污染 | 洗涤液现用现配，手工洗板悬空加洗液，不触及板孔 |
| | 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 |
| | 终止后读数不及时 | 加入终止液后立即读数 |
| | TMB 底物孵育在强光下进行 | 显色时避光孵育 |

## 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。

8、 本试剂盒未与其他厂家同类试剂盒或不同方法检测同一目的物的产品进行对比，所以不排除检测结果不一致的情况。

9、 试剂盒仅供研究使用，如将其用于临床诊断或任何其他用途，我公司将不对因此产生的问题负责，亦不承担任何法律责任。

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

## FineTest®

# Heme assay kit

Catalogue No.: EU2610

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

## Technical support related documents

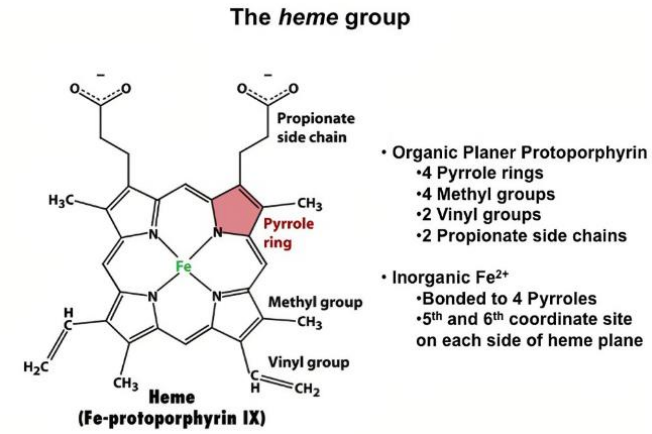
| | | |
|---|---|---|
| Title of Document | TMB color rendering control | Standard curve and concentration calculation software CurveExpert1.4(Including tutorial) |
| Website | https://www.fn-test.com/videos/targeted-control-of-tmb-coloring/ | https://www.fn-test.com/content/uploads/2019/08/CurveExpert-1.4.zip |
| Quick Mark | | |

## Product Features

| | | | |
|---|---|---|---|
| Application | For quantitative detection of heme in serum, plasma (e.g. jaundice or hemolysis blood test) and RBC lysate. | | |
| Reactivity | Universal | Detection Method | Enzyme catalysis |
| Range | 15.6-1000pmol/ml | Sensitivity | 5pmol/ml |
| Detection Duration | 20min (excluding balancing and sample preparation) | | |
| Samples needed for single well(Max) | Serum: 3ul, Plasma: 3ul, Cell Culture Supernatant: 100ul, cell or tissue lysate: 100ul, Other liquid samples: 50ul | | |
| Specificity | Specifically recognize Heme, no obvious cross reaction with other analogues | | |
| Storage | 2-8°C (for sealed box), please do not freeze! See kit label for expiry date | | |

## Background

Heme is a non-protein iron porphyrin component of hemoglobin, and also found in other respiratory pigments, animal/plant cells. As the ferrous derivative of protoporphyrin, heme is a kind of coordination chelate linked by 4 pyrrole rings and iron atom.Heme is the prosthetic group of hemoglobin and some oxidoreductases, involved in the oxygen transfer and oxido-reduction in the organism. Processed by a series of enzymes in the hepatocyte, liposoluble unconjugated bilirubin is converted into water-soluble conjugated bilirubin (direct bilirubin). Due to damaged red blood cells, increased liver load, disorders of hepatocyte transport, binding and excretion, or extrahepatic biliary obstruction, the increased bilirubin concentration in the blood can result in the jaundice.



## Principle of the Assay

This kit is based on enzyme assay method. Activated heme can oxidize TMB to blue solution(max absorption peak: 630nm). The kit offers heme standard and activation diluent buffer. The activated standard and diluted sample were added to the wells subsequently. TMB substrates were added into each well to to visualize the enzyme-substrate reaction(blue), which is stopped by adding sulfuric acid solution later. Read the O.D. absorbance at 450nm by spectrophotometry. Compare the OD450 value with standard curve using the curve fitting software, and then the concentration of heme in the sample can be calculated.

## Kit Components and Storage

The sealed kit can be stored at 2-8 ℃.

| Item | Specifications(48T/96T) | Storage |
|---|---|---|
| Microplate | 8×6/8×12 | RT |
| 10×Heme Standard(liquid) 10nmol/ml | 60ul/120ul | 2-8°C |
| Activation diluent buffer | 10ml/20ml | 2-8°C |
| TMB Substrate | 5ml/10ml | 2-8°C(Avoid Direct Light) |
| Stop Solution | 5ml/10ml | 2-8°C |
| Plate Sealer | 3/5pieces | |
| Product Description | 1copy | |

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

## Required Instruments and Reagents

1. Microplate reader (wavelength: 450nm)
2. 37°C incubator ($CO_2$ incubator for cell culture is not recommended.)
3. Automated plate washer or multi-channel pipette/5ml pipettor (for manual washing purpose)
4. Precision single (0.5-10μL, 5-50μL, 20-200μL, 200-1000μL) and multi-channel pipette with disposable tips(calibration is required before use.)
5. Sterile tubes and Eppendorf tubes with disposable tips
6. Absorbent paper and loading slot
7. Deionized or distilled water

## 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**

Generally 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 normal saline (0.9% NaCl). Then weigh for usage.

3.2. Use normal saline (0.9% NaCl) to grind tissue homogenates on the ice. The adding volume of normal saline (0.9% NaCl) depends on the weight of the tissue. Usually, 9mL normal saline (0.9% NaCl) would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS (e.g. 1mM PMSF).

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 Elisa assay should be within 1-3mg/ml.

**Note: The use of other lysates is not recommended, which can cause precipitation and non-color development during TMB incubation.**

**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 normal saline (0.9% NaCl) into collected cell and mix gently. Recollect cell by repeating centrifugation. Add 0.5-1ml normal saline (0.9% NaCl) and appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Disrupt the cell by ultrasonic disruption on ice. (3~5mm probe, 150-300W, 3~5 s/time, 30s intervals for 1~2s working)..

5.2. Adherent Cell Lysate: Absorb supernatant and add pre-cooling normal saline (0.9% NaCl) to wash three times. Add 0.5-1ml normal saline (0.9% NaCl) and appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Scrape the adherent cell with cell scraper. Disrupt the cell by ultrasonic disruption on ice. (3~5mm probe, 150-300W, 3~5 s/time, 30s intervals for 1~2s working)..

5.3. At the end of 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.

Recommended reagents for sample preparation: Cat No: E051 100mM PMSF protease inhibitor.

## 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.

5. Recombinant protein may not match with the capture or detection antibody in the kit, resulting in the undetectable assay.

## Precautions for Kits

1. When using different Elisa kits, labeling is required to avoid mixed components and failed assay.

2. After opening the kit, please refer to the table of storage condition for coated plate and 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 lyophilized standard)

3. 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.

4. While manual washing, please keep tips or pipettors for adding wash buffer away from the well. Insufficient washing or contamination easily causes false positive and high background.

5. During the assay, prepare required reagents for next step in advance. After washing, add the reagent into the well in time to avoid dryness. Otherwise, dry plate will result in the failed assay.

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

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

8. After loading, seal the plate to avoid the evaporation of the sample during incubation. Complete the incubation process at recommended temperature.

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

## Recommended Sample Dilution Ratio

Please refer to the following table of recommended dilution ratio for limited samples for reference. (ND: Not Detected)

| Sample Type | Recommended Dilution Ratio | Content |
|---|---|---|
| Human plasma (n=5) | 1/100-1/200 | 9-13 nmol/ml (umol/L) |
| Human serum (n=5) | 1/100-1/200 | 6-21 nmol/ml (umol/L) |
| Rat serum (n=5) | 1/100-1/400 | 20-63 nmol/ml (umol/L) |
| mouse serum (n=5) | 1/100-1/400 | 24-77 nmol/ml (umol/L) |
| mouse whole blood (n=5) | 1/10000-1/400000 | 10 (mmol/L) |

**The matrix components in serum/plasma will affect the test results, which it need to be diluted at least 1/2 with Sample Dilution Buffer before testing!**

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 60ul sample into 60ul sample diluent and mix gently.

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

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

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

For 50 fold dilution (1/50): One step dilution. Add 3ul sample and 47ul normal saline (0.9% NaCl) into 100ul sample diluent and mix gently.

For 100 fold dilution (1/100): One step dilution. Add 3ul sample and 177ul normal saline into 120ul sample diluent and mix gently.

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

For 10000 fold dilution (1/10000): Two step dilution. Create a 100-fold dilution first (normal saline is used throughout the dilution). 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 (normal saline is used in the first two steps.) Finally, create a 100-fold dilution and mix gently.

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

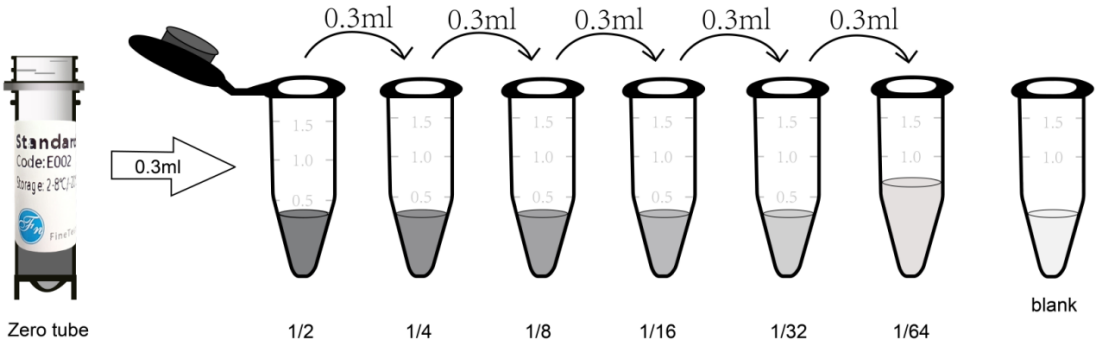
## Reagent Preparation and Storage

Take the Elisa kit from the fridge around 20 minutes earlier and equilibrate to room temperature(18-25℃).
For repeated assays, please just take the strips and standards required for the current assay, store the rest materials according to the relevant condition.

**1. Standards**

1.1. **Zero tube**: add 450ul Activation Diluent Buffer into one empty EP tube, then add 50ul 10×Heme standard and mix well(Working solution after preparation: 1x Heme standard, 1000pmol/ml).

1.2. 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 sample 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 sample dilution buffer. The standard concentration from zero tube to blank tube is 1000pmol/ml, 500pmol/ml, 250pmol/ml, 125pmol/ml, 62.5pmol/ml, 31.25pmol/ml, 15.625pmol/ml, 0pmol/ml.




Prepare standard solutions

Notes: Store the zero tube with dissolved standards at 2-8℃ and use it within 12h. Other diluted working solutions containing standards should be used in 2h.

## Assay Procedure

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test.

**Step 1:** Set standard, **test samples**, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate.

**Step 2: Add Samples:** Add 100ul of properly diluted sample or standard into relevant wells.

**Step 3: TMB Substrate:** Add 100ul TMB Substrate into each well, cover the plate and incubate at 37°C in dark for 20 minutes.

**Step 4: Stop:** Add 50ul Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.

**Step 5：** OD Measurement: Read the O.D. absorbance at 450nm in a microplate reader immediately. (If your microplate reader has a choice of correction wavelength, set it to 570nm or 630nm. Correct the read value to the OD450 value minus the OD570 or OD630 value. In this way, the OD value of non-chromogenic substances can be corrected and removed, thus obtaining more accurate results. If the microplate reader does not have a 570nm or 630nm wavelength, the original OD450 value can be used.)

## Calculation of Results

**（Operate Video：https://www.fn-test.com/videos/elisa-sample-concentration-calculation/）**

1. Calculate the mean OD450 value (using the original OD450 value or the corrected OD450 value) of the duplicate readings for each standard, control, and sample. Then, obtain the value of calculation by subtracting the OD450 blank.

2. Create a four parameter logistic curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. (Remove the OD450 blank during plotting.) 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).

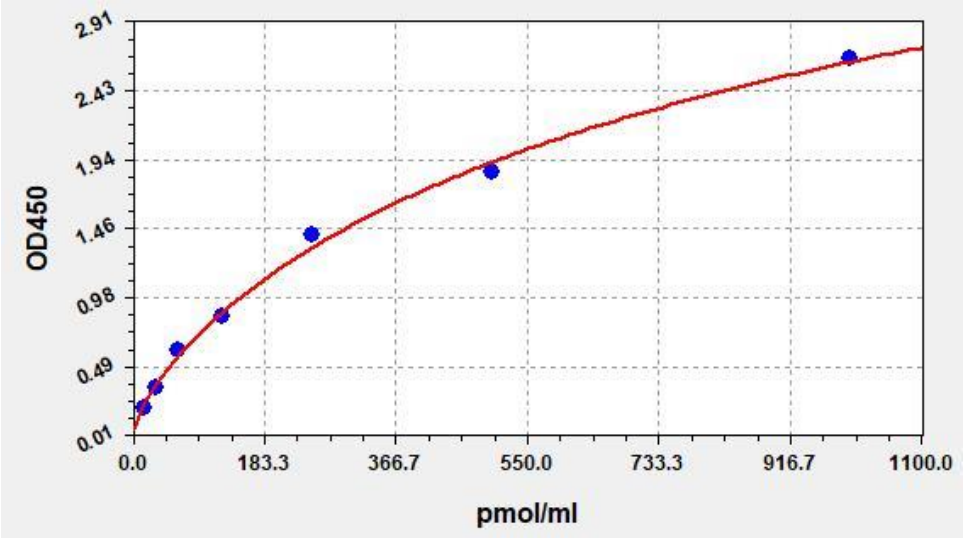
3. Calculate the sample concentration by substituting OD450 value into the standard curve. Diluted samples should be multiplied by the relevant dilution ratio.

## 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. TMB was balanced to 37°C before color development, and incubated at 37°C for 15 minutes in the dark after adding the enzyme label plate holes.)

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.(pmol/ml) | OD-1 | OD-2 | Average | Corrected |
|---|---|---|---|---|
| 0 | 0.069 | 0.071 | 0.07 | 0 |
| 15.625 | 0.206 | 0.212 | 0.209 | 0.139 |
| 31.25 | 0.341 | 0.351 | 0.346 | 0.276 |
| 62.5 | 0.597 | 0.615 | 0.606 | 0.536 |
| 125 | 0.834 | 0.858 | 0.846 | 0.776 |
| 250 | 1.401 | 1.441 | 1.421 | 1.351 |
| 500 | 1.829 | 1.883 | 1.856 | 1.786 |
| 1000 | 2.69 | 2.62 | 2.655 | 2.585 |



## 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 (pmol/ml) | 30.53 | 120.9 | 508 | 31.06 | 124.3 | 503 |
| Standard deviation | 1.49 | 5.42 | 24.38 | 1.36 | 5.84 | 21.83 |
| CV(%) | 4.87 | 4.48 | 4.8 | 4.38 | 4.7 | 4.34 |

## Recovery

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

| Matrix | Recovery Range (%) | Average (%) |
|---|---|---|
| Serum(n=5) | 88-98 | 94 |
| EDTA Plasma(n=5) | 89-100 | 95 |
| Heparin Plasma(n=5) | 95-105 | 99 |

## Linearity

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

| Sample | 1:2 | 1:4 | 1:8 |
|---|---|---|---|
| Serum(n=5) | 87-105% | 86-101% | 84-100% |
| EDTA Plasma(n=5) | 85-94% | 86-95% | 80-94% |
| Heparin Plasma(n=5) | 90-103% | 83-98% | 81-100% |

## Stability

Perform the stability test for the sealed kit at 37°C and 2-8°C and get relevant data.

| Elisa kit(n=5) | 37°C for 1 month | 2-8°C for 6 months |
|---|---|---|
| Average (%) | 80 | 95-100 |

## ELISA Troubleshooting

If the ELISA 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 | Incorrect order for adding reagents | Confirm the required reagent added in each step. Also repeat the assay and verify. |
| | Use components from different kits | Use the component included in the same kit. Also repeat the assay and verify. |
| | 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. |
| Poor standard curve | Inappropriate curve fitting model | Try to plot the curve by different fitting models. |
| 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 or freeze-thaw cycle | Aliquot and store samples according to the assay requirement. |
| High CV% | Precipitate is formed in the well during staining. | 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. |
| | Each well is washed unevenly. | Check whether the tube of the washer is smooth. |
| | 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 lyophilized 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 well is over dried. | The assay and sample loading process can't be terminated. Especially after washing the plate, add reagents immediately. Seal the plate during incubation. |
| | Slow colorimetric reaction | Before use, equilibrate the whole bottle of TMB substrate for 30min at 37°C. Extend the incubation time. |
| | The wavelength of the microplate reader is incorrect. | Check the wavelength and read the OD450 value again. |
| | The well is washed excessively. | Follow suggested washing times in this manual. |
| High Background | Insufficient washing | Follow suggested washing times in this manual. |
| | Wash buffer is contaminated. | Use the prepared wash buffer immediately. During manual washing, add wash buffer without touching the well. |
| | 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 adding the stop solution. |
| | TMB substrate is incubated in strong light. | During colorimetry, incubate in the dark. |

## 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.