（本试剂盒仅供体外研究使用，不用于临床诊断!）

产品货号：E-EL-H6013

产品规格：96T/48T/24T

人铁调素(Hepc)酶联免疫吸附测定试剂盒使用说明书

Human Hepc(Hepcidin) ELISA Kit

使用前请仔细阅读说明书。如果有任何问题，请通过以下方式联系我们：

销售部电话	027-65022280，027-87854967
技术部电话	027-87526315
电子邮箱（销售）	Perry@elabscience.cn
电子邮箱（技术）	techsupport@elabscience.cn
QQ 客服	800110755
网址	www.elabscience.cn

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。

联系时请提供产品批号(见试剂盒标签)，以便我们更高效地为您服务。

用途

该试剂盒用于体外定量检测人血清、血浆或其它相关生物液体中Hepc浓度。

灵敏度、检测范围、特异性和重复性

- 灵敏度：37.5pg/mL。
- 检测范围：62.5-4000pg/mL。
- 特异性：可检测样本中的人 Hepc,且与其它类似物无明显交叉反应。
- 重复性：板内，板间变异系数均<10%。

检测原理

本试剂盒采用双抗体夹心 ELISA 法。用抗人 Hepc 抗体包被于酶标板上，实验时样品（或标准品）中的人 Hepc 会与包被抗体结合，游离的成分被洗去。后依次加入生物素化的抗人 Hepc 抗体和辣根过氧化物酶标记的亲和素，抗人 Hepc 抗体与结合在包被抗体上的人 Hepc 结合，生物素与亲和素特异性结合而形成免疫复合物，游离的成分被洗去。加入显色底物(TMB)，TMB 在辣根过氧化物酶的催化下呈现蓝色，加终止液后变成黄色。用酶标仪在 450nm 波长处测 OD 值，Hepc 浓度与 OD_{450} 值之间呈正比，通过绘制标准曲线计算出样品中 Hepc 的浓度。

试剂盒组成及保存

未拆封的试剂盒可在 2-8℃保存一周；如果一周以后才使用试剂盒，请拆开试剂盒并按照下表中的条件分别保存各组分。

中文名称	规格	开封后保存条件
ELISA 酶标板 Micro ELISA Plate	96T：8 孔×12 条 48T：8 孔×6 条 24T：8 孔×3 条	-20℃，可存放 6 个月
冻干标准品 Reference Standard	96T：2 支 48T：1 支 24T：1 支	
浓缩生物素化抗体 (100×) Concentrated Biotinylated Detection Ab (100×)	96T：1 支 120μL 48T：1 支 60μL 24T：1 支 60μL	
浓缩 HRP 酶结合物（100×） Concentrated HRP Conjugate（100×）	96T：1 支 120μL 48T：1 支 60μL 24T：1 支 60μL	-20℃(避光) 可存放 6 个月
标准品&样品稀释液 Reference Standard & Sample Diluent	1 瓶 20mL	2-8℃，可存放 6 个月
生物素化抗体稀释液 Biotinylated Detection Ab Diluent	1 瓶 14mL	
酶结合物稀释液 HRP Conjugate Diluent	1 瓶 14mL	
浓缩洗涤液 (25×) Concentrated Wash Buffer (25×)	1 瓶 30mL	
底物溶液(TMB) Substrate Reagent	1 瓶 10mL	2-8℃(避光)
反应终止液 Stop Solution	1 瓶 10mL	2-8℃
封板覆膜 Plate Sealer	5 张	
产品说明书 Manual	1 份	
质检报告 Certificate of Analysis	1 份	

说明：所有试剂瓶盖须旋紧以防止蒸发和微生物的污染。
试剂体积以实际发货版说明书为准。相关试剂在分装时会比标签上标明的体积稍多一些，请在使用时量取而非直接倒出。

试验所需自备物品

1. 酶标仪(450nm波长滤光片)
2. 高精度移液器，EP管及一次性吸头：0.5-10μL，2-20μL，20-200μL，200-1000μL
3. 37℃恒温箱，双蒸水或去离子水
4. 吸水纸
5. 加样槽

注意事项

1. 试验中请穿着实验服并戴乳胶手套做好防护工作。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。
2. 刚开启的酶标板孔中可能会有少许水样物质，此为正常现象，不会对实验结果造成任何影响。暂时不用的板条应拆卸后放入备用铝箔袋，按照上述表格中保存条件存放。
3. 请勿重复使用已稀释过的标准品、生物素化抗体工作液、酶结合物工作液。未用完的浓缩生物素化抗体(100×)、浓缩HRP酶结合物(100×)、酶标板及其他原液按照上述表格中保存条件存放。
4. 检测使用的酶标仪需要安装能检测450±10nm波长的滤光片，光密度范围在0-3.5之间。建议使用时提前15分钟预热。
5. 不同批号的试剂盒组份不能混用。
6. 试验中所用的EP管和吸头均为一次性使用，严禁混用。

样品收集方法

(具体处理方法可参考官网：http://www.elabscience.cn/List-detail-241.html)

1. 血清：全血样品于室温放置2小时或2-8℃过夜后于2-8℃，1000×g离心20分钟，取上清即可检测。
2. 血浆：抗凝剂推荐使用EDTA-Na_2，样品采集后30分钟内于2-8℃，1000×g离心15分钟，取上清即可检测。
3. 组织匀浆：用预冷的PBS (0.01M, pH=7.4)冲洗组织，去除残留血液，称重后将组织剪碎。将剪碎的组织与对应体积的PBS(一般按1:9的重量体积比，比如1g的组织样品对应9mL的PBS，具体体积可根据实验需要适当调整，并做好记录。推荐在PBS中加入蛋白酶抑制剂)加入玻璃匀浆器中，在冰上充分研磨。为了进一步裂解组织细胞，可以对匀浆液进行反复冻融或超声破碎。最后将匀浆液于2-8℃，5000×g离心5-10分钟，取上清检测。
4. 细胞提取液：贴壁细胞用冷的PBS轻轻清洗，然后用胰蛋白酶消化，1000×g离心5分钟后收集细胞；悬浮细胞可直接离心收集。收集的细胞用冷的PBS洗涤3次。每10^6个细胞中加入150-200μL PBS重悬(推荐在PBS中加入蛋白酶抑制剂；若含量很低可减少PBS的体积)并通过反复冻融或超声使细胞破

碎。将提取液于2-8℃，1500×g离心10分钟，取上清检测。
5. 细胞培养上清或其他生物体液：收集液体后于2-8℃，1000×g离心20分钟，除去杂质及细胞碎片。取上清检测。

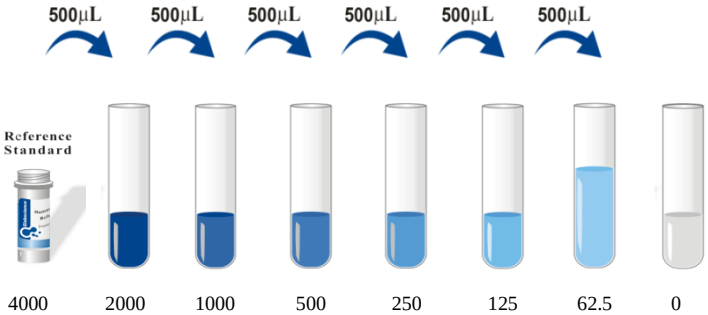
样品注意事项

1. 收集血液的试管应为一次性的无内毒素试管。避免使用溶血，高血脂样品。
2. 样品收集后若在1周内进行检测可保存于2-8℃，若不能及时检测，请按一次使用量分装，冻存于-20℃(1个月内检测)，或-80℃(3个月内检测)，避免反复冻融。在检测前，冷冻过的样本应缓慢地融化并离心除去冻融过程产生的沉淀物。室温混匀后使用。
3. 试剂盒检测范围不等同于样本中待测物的浓度范围，建议实验前通过相关文献预估样本中待测物的浓度并通过预实验确定样本的实际浓度情况。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. **正常血清/血浆样本推荐稀释20~100倍。**参考稀释方案如下：
   稀释100倍：一步稀释。取5μL样本到495μL标准品&样本稀释液内，做100倍稀释；
   稀释1000倍：两步稀释。取5μL样本到95μL标准品&样本稀释液内，做20倍稀释，再取5μL 20倍稀释样本到245μL标准品&样本稀释液内，做50倍稀释，总共稀释1000倍；
   稀释100000倍：三步稀释。取5μL样本到195μL标准品&样本稀释液内，做40倍稀释，再取5μL 40倍稀释样本到245μL标准品&样本稀释液内，做50倍稀释，最后取5μL 2000倍稀释样本到245μL标准品&样本稀释液内，做50倍稀释，总共稀释100000倍；
   每步稀释时取液量不少于3μL，稀释倍数不超过100倍。每步稀释都需混合均匀，避免起泡。
5. 若所检样本不在说明书所列样本之中，建议做预实验验证其检测有效性。
6. 若使用化学裂解液制备组织匀浆或细胞提取液，由于引入某些化学物质会导致ELISA测值出现偏差。
7. 某些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。

检测前准备工作

1. 提前20分钟从冰箱中取出试剂盒，平衡至室温(18-25℃)。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和试剂，剩余板条和试剂需按照指定条件保存。
2. **洗涤液：**将**浓缩洗涤液**用双蒸水稀释(1:24)。提示：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象，可用40℃水浴微加热使结晶完全溶解后再配制洗涤液。当日使用。

3. **标准品工作液：**将**标准品**于10000×g离心1分钟，加入**标准品&样品稀释液**1.0mL至冻干标准品中，旋紧管盖，静置10分钟，上下颠倒数次，待其充分溶解后，轻轻混匀，避免起泡，配成4000pg/mL的标准品工作液(或加入1.0mL**标准品&样品稀释液**后，静置1-2分钟，用低速涡旋仪充分混匀。可通过低速离心去除涡旋过程中产生的气泡)。然后根据需要进行倍比稀释。建议配制成以下浓度：4000、2000、1000、500、250、125、62.5、0pg/mL。倍比稀释方法：取7支EP管，每管中加入500μL**标准品&样品稀释液**，从4000pg/mL的标准品工作液中吸取500μL到其中一支EP管中混匀配成2000pg/mL的标准品工作液，按此步骤往后依次吸取混匀。如下图。提示：最后一管直接作为空白孔，不需要再从倒数第二管中吸取液体。



4. **生物素化抗体工作液：**实验前计算当次实验所需用量(以100μL/孔计算)，实际配制时应多配制100-200μL。使用前15分钟，将**浓缩生物素化抗体**于800×g离心1分钟，以**生物素化抗体稀释液**将100×**浓缩生物素化抗体**稀释成1×工作浓度(例如：10μL浓缩液+990μL稀释液)。当日使用。
5. **酶结合物工作液：**实验前计算当次实验所需用量(以100μL/孔计算)，实际配制时应多配制100-200μL。使用前15分钟，将**浓缩HRP酶结合物**于800×g离心1分钟，以**酶结合物稀释液**将100×**浓缩HRP酶结合物**稀释成1×工作浓度(例如：10μL浓缩液+990μL稀释液)。当日使用。

操作步骤(第11页中附有简版操作概要)

1. 分别设定**标准孔**、**空白孔**和**样本孔**。标准孔加入 100μL 倍比稀释的标准品，空白孔加入 100μL 标准品&样本稀释液，其余孔加入 100μL 待测样本(建议所有的待检样本和标准品在检测中设立复孔。**试剂盒检测范围不等同于样本中待测物的浓度范围，若样本浓度高于检测范围，需用标准品&样本**

稀释液稀释后取样)。给酶标板覆膜，37℃孵育 90 分钟。提示：加样时将样品加于酶标板底部，尽量不触及孔壁，轻轻晃动混匀，避免产生气泡。加样时间宜控制在 10 分钟内。

2. 甩尽孔内液体，不用洗涤。每个孔中加入**生物素化抗体工作液** 100μL，酶标板加上覆膜，37℃温育 1 小时。
3. 甩尽孔内液体，每孔加**洗涤液** 350μL，浸泡 1-2 分钟，吸去或甩掉酶标板内的液体，在厚的吸水纸上拍干。重复此洗板步骤 3 次。提示：此处与其他洗板步骤都可用洗板机(参考北京拓普 DEM-3 型洗板机参数设置：2 点吸，每孔加入洗涤液 350μL，振板 5 秒，吸液 0.5 秒)。洗板完成后请立即进行下步操作，不要让微孔板干燥。
4. 每孔加**酶结合物工作液** 100μL，酶标板加上覆膜，37℃温育 30 分钟。
5. 甩尽孔内液体，洗板 5 次，方法同步骤 3。
6. 每孔加**底物溶液(TMB)**90μL，酶标板加上覆膜，37℃避光孵育 15 分钟左右。提示：根据实际显色情况酌情缩短或延长，但不可超过 30 分钟。当标准孔出现明显梯度时(标准孔前 4 孔出现明显蓝色梯度,后 3-4 孔不明显),即可终止。提前 15 分钟打开酶标仪预热。
7. 每孔加**终止液** 50μL，终止反应。提示：终止液的加入顺序应尽量与底物溶液的加入顺序相同。
8. 立即用酶标仪在 450nm 波长测量各孔的光密度(OD 值)。

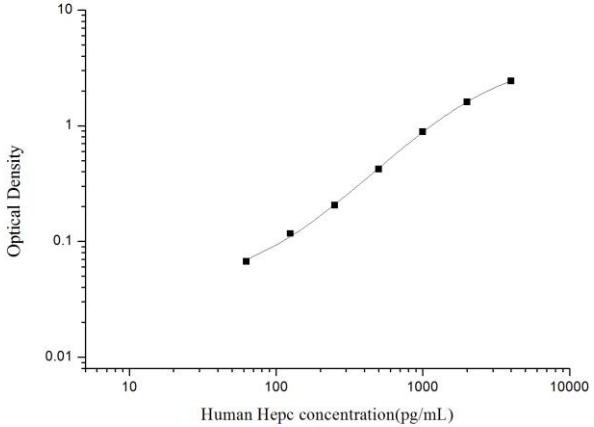
结果判断

1. 计算标准品和样本复孔的平均OD值并减去空白孔的OD值作为校正值。以浓度为横坐标，OD值为纵坐标，在双对数坐标纸上绘出四参数逻辑函数的标准曲线(作图时去掉空白组的值)。
2. 若样品OD值高于标准曲线上限，应适当稀释后重测并在计算样本浓度时乘以相应的稀释倍数。

典型数据

以下数据和曲线仅供参考，实验者需根据自己的实验建立标准曲线。

浓度(pg/mL)	4000	2000	1000	500	250	125	62.5	0
OD	2.495	1.659	0.948	0.481	0.263	0.175	0.125	0.058
校正 OD	2.437	1.601	0.89	0.423	0.205	0.117	0.067	-



精密度

板内精密度:低浓度样本,中浓度样本和高浓度样本分别在1块板子上检测20次。板间精密度:低浓度样本,中浓度样本和高浓度样本分别在3块板子上检测20次。

	批内变异系数			批间变异系数		
样本	1	2	3	1	2	3
数量	20	20	20	20	20	20
平均值(pg/mL)	199.97	497.72	1601.67	192.8	452.62	1807.22
标准差	10.7	24.59	65.83	9.79	22.45	91.81
变异系数(%)	5.35	4.94	4.11	5.08	4.96	5.08

回收率

分别往不同样本中添加已知浓度的人Hepc，做回收实验，得出回收率范围和平均回收率。

样本类型	回收率范围 (%)	平均回收率 (%)
血清(n=8)	94-105	101
血浆(EDTA)(n=8)	88-100	94
细胞培养基(n=8)	95-111	103

线性

将添加有人Hepc的样本分别稀释2倍，4倍，8倍，16倍做回收实验，得出回收率范围及平均回收率。

		血清 (n=5)	血浆 (EDTA) (n=5)	细胞培养基 (n=5)
1:2	回收率范围(%)	87-99	93-104	90-105
	平均回收率(%)	94	101	97
1:4	回收率范围(%)	92-103	98-109	82-94
	平均回收率(%)	98	101	90
1:8	回收率范围(%)	101-110	91-106	99-109
	平均回收率(%)	104	99	103
1:16	回收率范围(%)	91-102	88-104	89-98
	平均回收率(%)	94	96	95

问题分析

若实验效果不好，请及时对显色结果拍照，保存实验数据，保留所用板条及未使用试剂，然后联系我公司技术支持为您解决问题。同时您也可以参考以下资料：

问题描述	可能原因	相应对策
标准曲线梯度差	吸液或加液不准	检查移液器及吸头
	标准品稀释不正确	溶解标准品时稍微旋转瓶身，轻轻混匀使粉末完全
	洗涤不完全	保证洗涤时间和洗涤次数及每孔的加液量
显色很弱或无色	孵育时间太短	保证充足的孵育时间
	实验温度不正确	使用推荐的实验温度
	试剂体积不够或漏加	检查吸液及加液过程，保证所有试剂按顺序足量添加
	稀释不正确	
	酶标记物失活或底物失效	混合酶结合物和底物，通过迅速显色来检查判断
读数数值低	酶标仪设置不正确	在酶标仪上检查波长及滤光片设置
		提前打开酶标仪预热
变异系数大	加液不正确	检查加液情况
背景值高	检测抗体的工作浓度过高	使用推荐的稀释倍数
	酶标板洗涤不完全	保证每步清洗完全；如果用自动洗板机，请检查所有的出口是否有堵塞；是否使用试剂盒配备的洗涤液
	洗液有污染	配制新鲜的洗液
灵敏度低	ELISA 试剂盒保存不	按说明书要求保存相关试剂
	读数前未终止	OD 读数前应在每孔中加入终止液

操作概要

1. 在各孔中加入标准品或样品各 100μL，37℃孵育 90 分钟

2. 倒去孔内液体，加入 100μL 生物素化抗体工作液，37℃孵育 60 分钟

3. 洗涤 3 次

4. 加入 100μL 酶结合物工作液， 37℃孵育 30 分钟

5. 洗涤 5 次

6. 加入 90μL 底物溶液， 37℃孵育 15 分钟左右

7. 加入 50μL 终止液，立即在 450nm 波长处测量 OD 值

8. 结果计算

声明

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Human Hepc(Hepcidin) ELISA Kit

Catalog No: E-EL-H6013
Size: 96T/48T/24T

Intended use

This ELISA kit applies to the in vitro quantitative determination of Human Hepc concentrations in serum, plasma and other biological fluids.

Specification

- Sensitivity: 37.5pg/mL.
- Detection Range: 62.5-4000pg/mL.
- Specificity: This kit recognizes Human Hepc in samples. No significant cross-reactivity or interference between Human Hepc and analogues was observed.
- Repeatability: Coefficient of variation is < 10%.

Test principle

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human Hepc. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human Hepc and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human Hepc, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional to the concentration of Human Hepc. You can calculate the concentration of Human Hepc in the samples by comparing the OD of the samples to the standard curve.

Kit components & Storage

An unopened kit can be stored at 2-8℃ for 1 week. If the kit is not supposed to be used within 1 week, store the items separately according to the following conditions once the kit is received.

Item	Specifications	Storage
Micro ELISA Plate (Dismountable)	96T: 8 wells ×12 strips 48T: 8 wells ×6 strips 24T: 8 wells ×3 strips	-20℃, 6 months
Reference Standard	96T: 2 vials 48T: 1 vial 24T: 1 vial	
Concentrated Biotinylated Detection Ab(100×)	96T: 1 vial, 120 μL 48T: 1 vial, 60 μL 24T: 1 vial, 60 μL	
Concentrated HRP Conjugate (100×)	96T: 1 vial, 120 μL 48T: 1 vial, 60 μL 24T: 1 vial, 60 μL	-20℃(Protect from light), 6 months
Reference Standard & Sample Diluent	1 vial, 20 mL	2-8℃, 6 months
Biotinylated Detection Ab Diluent	1 vial, 14 mL	
HRP Conjugate Diluent	1 vial, 14 mL	
Concentrated Wash Buffer(25×)	1 vial, 30 mL	
Substrate Reagent	1 vial, 10 mL	2-8℃(Protect from light)
Stop Solution	1 vial, 10 mL	2-8℃
Plate Sealer	5 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution. The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

Other supplies required

Microplate reader with 450nm wavelength filter
High-precision transfer pipette, EP tubes and disposable pipette tips
Incubator capable of maintaining 37℃
Deionized or distilled water
Absorbent paper
Loading slot

Note

1. Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
2. A freshly opened ELISA plate may appear a water-like substance, which is normal and will not have any impact on the experimental results. Return the unused wells to the foil pouch provided in the kit, store it according to the conditions suggested in the above table.
3. Do not reuse the reconstituted standard, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab (100×) and other stock solutions should be stored according to the storage conditions in the above table.
4. The microplate reader should be able to be installed with a filter that can detect the wave length at 450±10 nm. The optical density should be within 0-3.5. Follow the Instructions of the Microplate Reader for set-up and preheat it for 15 min before OD measurement.
5. Do not mix or use components with those from other lots.
6. Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.

Sample collection

(More detailed information please view our website: http://www.elabscience.cn/List–detail-241.html)

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 2-8℃ before centrifugation for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

Plasma: Collect plasma using EDTA-Na_2 as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8℃ within 30 min of collection. Collect the supernatant to carry out the assay.

Tissue homogenates: It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolyzed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5-10 min at 5000×g at 2-8℃ to get the supernatant.

Cell lysates: For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000×g. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1×10^6 cells, add 150-250 μL of pre-cooled PBS to keep the cells suspended. Repeat the freeze-thaw process several times or use an ultrasonic cell disrupter until the cells are fully lysed. Centrifuge for 10min at 1500×g at 2-8℃. Remove the cell fragments, collect the supernatant to carry out the assay.

Cell culture supernatant or other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

Note for sample

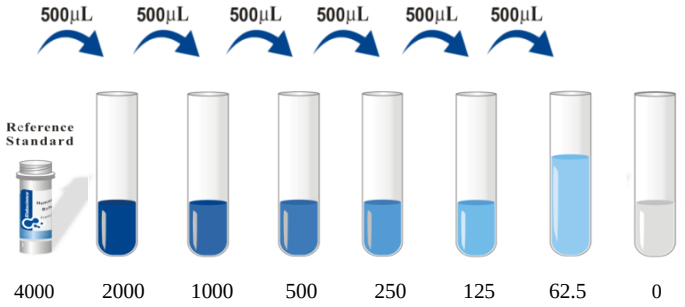
1. Tubes for blood collection should be disposable and be non-endotoxin. Samples with high hemolysis or much lipid are not suitable for ELISA assay.
2. Samples should be assayed within 7 days when stored at 2-8℃, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.
3. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
4. **It is suggested to dilute normal serum/plasma samples at 20~100 fold**. Please refer to the following dilution instructions:
   For 100 fold dilution: One-step dilution. Add 5μL sample to 495μL sample diluent to yield 100 fold dilution.
   For 1000 fold dilution: Two-step dilution. Add 5μL sample to 95μL sample diluent to yield 20 fold dilution, then add 5μL 20 fold diluted sample to 245μL sample diluent, after this, the neat sample has been diluted at 1000 fold successfully.
   100000 fold dilution: Three-step dilution. Add 5μL sample to 195μL sample diluent to yield 40 fold dilution, then add 5μL 40 fold diluted sample to 245μL sample diluent to yield 50 fold dilution, and finally add 5μL 2000 fold diluted sample to 245μL sample diluent, after this, the neat sample has been diluted at 100000 fold successfully.
5. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
6. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance.

7. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

Reagent preparation

1. Bring all reagents to room temperature (18-25℃) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
2. **Wash Buffer:** Dilute 30mL of Concentrated Wash Buffer with 720mL of deionized or distilled water to prepare 750mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 4000pg/mL(or add 1.0mL of Reference Standard &Sample Diluent, let it stand for 1-2 min and then mix it thoroughly with a vortex meter of low speed. Bubbles generated during vortex could be removed by centrifuging at a relatively low speed). Then make serial dilutions as needed. The recommended dilution gradient is as follows: 4000、2000、1000、500、250、125、62.5、0pg/mL.
   Dilution method: Take 7 EP tubes, add 500μL of Reference Standard & Sample Diluent to each tube. Pipette 500μL of the 4000pg/mL working solution to the first tube and mix up to produce a 2000pg/mL working solution. Pipette 500μL of the solution from the former tube into the latter one according to this step. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube. (The operation diagram is shown on the next page)
4. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (100μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated Biotinylated Detection Ab at 800×g for 1 min, then dilute the 100× Concentrated Biotinylated Detection Ab to 1× working solution with Biotinylated Detection Ab Diluent(Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent= 1: 99).
5. **HRP Conjugate working solution:** Calculate the required amount before the experiment (100μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate at 800×g for 1 min, then

dilute the 100× Concentrated HRP Conjugate to 1× working solution with HRP Conjugate Diluent(Concentrated HRP Conjugate: HRP Conjugate Diluent= 1: 99).



Assay procedure (A brief assay procedure is on the 23th page)

1. Determine wells for diluted standard, blank and sample. Add 100μL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Decant the liquid from each well, do not wash. Immediately add 100 μL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 1 hour at 37 ℃.
3. Decant the solution from each well，add 350μL of **wash buffer** to each well. Soak for 1-2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.
4. Add 100μL of **HRP Conjugate working solution** to each well. Cover the plate with a new sealer. Incubate for 30 min at 37 ℃.
5. Decant the solution from each well, repeat the wash process for 5 times as conducted in step 3.
6. Add 90μL of **Substrate Reagent** to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37 ℃. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not

more than 30min. Preheat the Microplate Reader for about 15 min before OD measurement.

7. Add 50μL of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Calculation of results

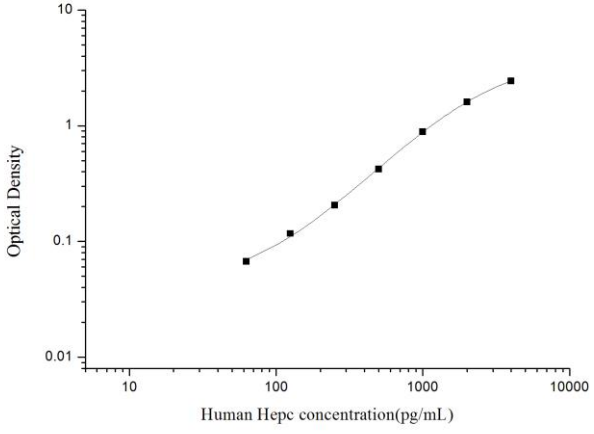
Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.

Concentration(pg/mL)	4000	2000	1000	500	250	125	62.5	0
OD	2.495	1.659	0.948	0.481	0.263	0.175	0.125	0.058
Corrected OD	2.437	1.601	0.89	0.423	0.205	0.117	0.067	-



Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level Human Hepc were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level Human Hepc were tested on 3 different plates, 20 replicates in each plate.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean(pg/mL)	199.97	497.72	1601.67	192.8	452.62	1807.22
Standard deviation	10.7	24.59	65.83	9.79	22.45	91.81
CV (%)	5.35	4.94	4.11	5.08	4.96	5.08

Recovery

The recovery of Human Hepc spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=8)	94-105	101
EDTA plasma (n=8)	88-100	94
Cell culture media(n=8)	95-111	103

Linearity

Samples were spiked with high concentrations of Human Hepc and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

		Serum (n=5)	EDTA plasma (n=5)	Cell culture media(n=5)
1:2	Range (%)	87-99	93-104	90-105
	Average (%)	94	101	97
1:4	Range (%)	92-103	98-109	82-94
	Average (%)	98	101	90
1:8	Range (%)	101-110	91-106	99-109
	Average (%)	104	99	103
1:16	Range (%)	91-102	88-104	89-98
	Average (%)	94	96	95

Troubleshooting

If the results are not good enough, please take pictures and save the experimental data in time. Keep the used plate and remaining reagents. Then contact our technical support to solve the problem. Meanwhile, you could also refer to the following materials:

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing.
	Wells are not completely aspirated	Completely aspirate wells in between steps.
Low signal	Insufficient incubation time	Ensure sufficient incubation time.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation.
	Improper dilution	
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB, rapid coloring.
Deep color but low value	Plate reader setting is not optimal	Verify the wavelength and filter setting on the Microplate reader.
		Open the Microplate Reader ahead to pre-heat.
Large CV	Inaccurate pipetting	Check pipettes.
High background	Concentration of target protein is too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions.
	Stop solution is not added	Stop solution should be added to each well before measurement.

SUMMARY

1. Add 100 µL standard or sample to each well. Incubate for 90 min at 37℃.

2. Remove the liquid. Add 100 µL Biotinylated Detection Ab. Incubate for 1 hour at 37℃.

3. Aspirate and wash 3 times.

4. Add 100 µL HRP Conjugate. Incubate for 30 min at 37℃.

5. Aspirate and wash 5 times.

6. Add 90 µL Substrate Reagent. Incubate for 15 min at 37℃.

7. Add 50 µL Stop Solution. Read at 450 nm immediately.

8. Calculation of results.

Declaration

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.
3. The final experimental results will be closely related to the validity of products, operational skills of the operators, the experimental environments and so on. We are only responsible for the kit itself, but not for the samples consumed during the assay. The users should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
4. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions.
5. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
6. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
7. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipment, and so on. Intra-assay variance among kits from different batches might arise from the above reasons too.
8. Kits from different manufacturers or other methods for testing the same analyte could bring out inconsistent results, since we haven't compared our products with those from other manufacturers.
9. The kit is designed for research use only, we will not be responsible for any issues if the kit is applied in clinical diagnosis or any other related procedures.