（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-H0119c
产品规格：96T/48T/24T/96T*5

Elabscience® 人肾素(REN)酶联免疫吸附测定试剂盒使用说明书
Human REN(Renin) 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	027-87879180，027-87854967
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
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。

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用途

该试剂盒用于体外定量检测人 血清、血浆或其他相关生物液体中REN浓度。

基本性能

性能	
灵敏度	18.75 pg/mL
检测范围	31.25-2000 pg/mL
特异性	可检测样本中的人REN，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REN抗体包被于酶标板上，实验时样品或标准品中的人REN会与包被抗体结合，游离的成分被洗去。依次加入生物素化的抗人REN抗体和辣根过氧化物酶标记的亲和素。抗人REN抗体与结合在包被抗体上的人REN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，REN浓度与OD450值之间呈正比，通过绘制标准曲线计算出样品中REN的浓度。

试剂盒组成及保存

未拆封的试剂盒可在2-8°C保存一周；如果一周以后才使用试剂盒，请拆开试剂盒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 96T*5：5 块 96T酶标板	-20°C，可存放6个月
冻干标准品 Reference Standard	96T：2 支 48T/24T：1 支 96T*5：10 支	
浓缩生物素化抗体 (100×) Concentrated Biotinylated Detection Ab (100×)	96T：1支 120 μL 48T/24T：1支 60 μL 96T*5：5 支 120 μL	
浓缩HRP酶结合物（100×） Concentrated HRP Conjugate(100×)	96T：1支 120 μL 48T/24T：1支 60 μL 96T*5：5 支 120 μL	-20°C(避光)，可存放6个月
标准品&样品稀释液 Reference Standard & Sample Diluent	96T/48T/24T：1瓶 20 mL 96T*5：5瓶 20 mL	2-8°C，可存放6个月
生物素化抗体稀释液 Biotinylated Detection Ab Diluent	96T/48T/24T：1瓶 14 mL 96T*5：5瓶 14 mL	
酶结合物稀释液 HRP Conjugate Diluent	96T/48T/24T：1瓶 14 mL 96T*5：5瓶 14 mL	
浓缩洗涤液 (25×) Concentrated Wash Buffer (25×)	96T/48T/24T：1瓶 30 mL 96T*5：5瓶 30 mL	
底物溶液(TMB) Substrate Reagent	96T/48T/24T：1瓶 10 mL 96T*5：5瓶 10 mL	2-8°C(避光)
反应终止液 Stop Solution	96T/48T/24T：1瓶 10 mL 96T*5：5瓶 10 mL	2-8°C
封板覆膜 Plate Sealer	96T/48T/24T：5张 96T*5：25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：0.5-10 μL, 2-20 μL, 20-200 μL, 200-1000 μL
3. 37℃恒温箱
4. 双蒸水或去离子水
5. 吸水纸
6. 加样槽

样品收集方法

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

1. **血清：**全血样品于室温放置1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-Na2，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1 g的组织样品对应9 mL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150-200 μL 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。
6. **样本处理相关试剂推荐：**10×EDTA抗凝剂 (货号：E-EL-SR003)、PMSF蛋白酶抑制剂 (货号：E-EL-SR002)、0.25%胰蛋白酶溶液 (货号：EP-CM-L0043)。

注意事项

■ 试剂盒注意事项

1) 本试剂盒仅供体外研究使用，不用于临床诊断。
2) 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。
3) 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。
4) 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。
5) 检测使用的酶标仪需要安装能检测450±2nm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。
6) 请勿使用其他批号或其他来源的试剂混合或替代本试剂盒中的试剂。
7) 试验中所用的EP管和吸头均为一次性使用，严禁混用。
8) 请勿使用过期的试剂。

■ 样品注意事项

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。如果样品中待测物浓度过高或者过低，请对样本做适当的稀释或者浓缩。
4) 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。
5) 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。
6) 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。

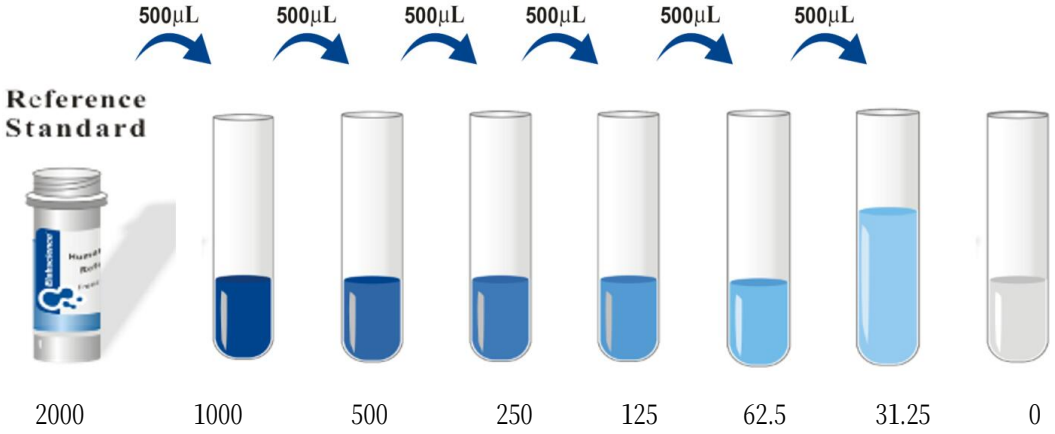
样本稀释方案

请提前预估样本的浓度范围，并通过预实验或咨询技术支持确定待检样品的稀释倍数。

如果您的待测样本需要稀释，通用稀释方案参考如下：
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**3 μL**，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 mL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2000pg/mL的标准品工作液(或加入1 mL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：2000,1000,500,250,125,62.5,31.25,0 pg/mL。倍比稀释方法：取7支 EP管，每管中加入500 μL标准品&样品稀释液,从2000pg/mL的标准品工作液中吸取500 μL到第一支EP管中混匀配成1000pg/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。倍比稀释的标准品工作液需要现配现用。

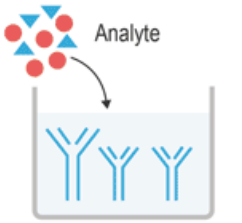


4. 生物素化抗体工作液：实验前计算当次实验所需用量(以100 μL/孔计算)，实际配制时应多配制100-200 μL。使用前15分钟，将浓缩生物素化抗体于800×g离心1分钟，以生物素化抗体稀释液将100×浓缩生物素化抗体稀释成1×工作浓度(例如：10 μL浓缩液+990 μL稀释液)。现配现用。
5. HRP酶结合物工作液：HRP酶结合物为HRP酶结合亲和素。实验前计算当次实验所需用量(以100 μL/孔计算)，实际配制时应多配制100-200 μL。使用前15分钟，将浓缩HRP酶结合物于800×g离心1分钟，以酶结合物稀释液将100×浓缩HRP酶结合物稀释成1×工作浓度(例如：10 μL浓缩液+990 μL稀释液)。现配现用。

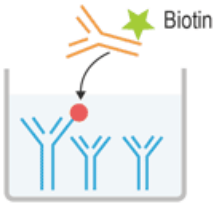
操作步骤

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；建议通过预实验或咨询技术支持确定待检样本的稀释倍数)。给酶标板覆膜，37°C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°C温育1小时。
3. 甩尽孔内液体，在洁净的吸水纸上拍干。每孔加洗涤液350μL，浸泡1分钟，吸去或甩掉酶标板内的液体，拍干。重复此洗板步骤3次。提示：此处与其他洗板步骤都可使用洗板机(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100 μL，酶标板加上覆膜，37°C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)90 μL，酶标板加上覆膜，37°C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(前4个显色孔出现明显蓝色梯度)，即可终止。提前15分钟打开酶标仪预热。
7. 每孔加终止液50 μL，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450 nm波长测量各孔的光密度(OD值)。

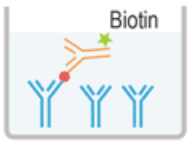
操作一览表



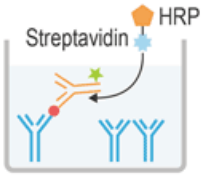
1. 对应板孔中加入100μL标准品工作液或样本，37℃孵育90分钟



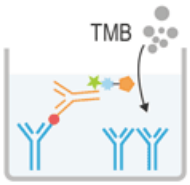
2. 弃掉板内液体后，立即加入100μL生物素化抗体工作液，37℃孵育60分钟



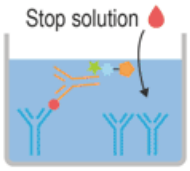
3. 弃掉板内液体，洗板3次



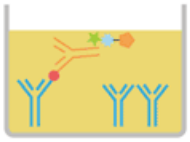
4. 每孔加入100μL HRP酶结合物工作液，37℃孵育30分钟，弃掉板内液体，洗板5次



5. 每孔加入90μL底物溶液，37℃孵育15分钟左右



6. 每孔加入50μL终止液



7. 立即在450nm波长下读数，处理数据

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，在双对数坐标轴上拟合四参数逻辑函数的标准曲线。
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。

技术资源

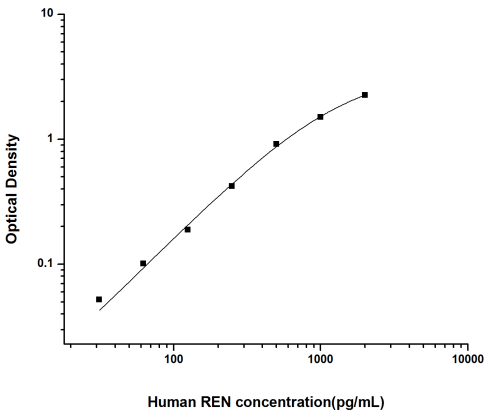
微信扫描左下二维码，获得更详细的ELISA实验指南和常规问题分析。
如有任何技术问题，请与我司技术支持联系(建议及时对显色结果拍照，保留实验数据、所用板条及未使用的试剂)。

ELISA实验指南

技术支持微信

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。

pg/mL	OD	校正OD	标曲
2000	2.315	2.262	
1000	1.552	1.499	
500	0.966	0.913	
250	0.473	0.42	
125	0.242	0.189	
62.5	0.154	0.101	
31.25	0.105	0.052	
0	0.053	-	

性能

■ 精密度

板内精密度：低，中，高浓度样本分别在1块板子上检测20次。
板间精密度：低，中，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)	108.90	258.70	836.60	98.90	234.70	771.40
标准差	5.90	11.90	36.80	6.40	10.80	28.50
变异系数 (%)	5.42	4.60	4.40	6.47	4.60	3.69

■ 回收率

分别往不同样本中添加已知浓度的人REN，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)	86-98	92
血浆(EDTA)(n=8)	84-98	90
细胞培养基(n=8)	83-96	90

■ 线性

将添加有人REN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(%)	98-111	96-107	98-110
	平均回收率(%)	104	101	104
1:4	回收率范围(%)	94-106	86-98	97-114
	平均回收率(%)	100	93	104
1:8	回收率范围(%)	99-112	82-95	93-107
	平均回收率(%)	106	88	100
1:16	回收率范围(%)	98-113	86-101	93-106
	平均回收率(%)	106	93	98

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。
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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。

Human REN(Renin) ELISA Kit
Catalog No : E-EL-H0119c
Size:96T/48T/24T/96T*5

Intended use

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

Character

Item	
Sensitivity	18.75 pg/mL
Detection Range	31.25-2000 pg/mL
Specificity	This kit recognizes Human REN in samples. No significant cross-reactivity or interference between Human REN 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 REN. 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 REN 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 REN, 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 nm ± 2 nm. The OD value is proportional to the concentration of Human REN. You can calculate the concentration of Human REN 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 96T*5: 5 plates, 96T	-20℃, 6 months
Reference Standard	96T: 2 vials 48T/24T: 1 vial 96T*5: 10 vials	
Concentrated Biotinylated Detection Ab (100×)	96T: 1 vial, 120 μL 48T/24T: 1 vial, 60 μL 96T*5: 5 vials, 120 μL	
Concentrated HRP Conjugate (100×)	96T: 1 vial, 120 μL 48T/24T: 1 vial, 60 μL 96T*5: 5 vials, 120 μL	-20°C(shading light), 6 months
Reference Standard & Sample Diluent	96T/48T/24T: 1 vial, 20 mL 96T*5: 5 vials, 20 mL	2-8°C, 6 months
Biotinylated Detection Ab Diluent	96T/48T/24T: 1 vial, 14 mL 96T*5: 5 vials, 14 mL	
HRP Conjugate Diluent	96T/48T/24T: 1 vial, 14 mL 96T*5: 5 vials, 14 mL	
Concentrated Wash Buffer (25×)	96T/48T/24T: 1 vial, 30 mL 96T*5: 5 vials, 30 mL	
Substrate Reagent	96T/48T/24T: 1 vial, 10 mL 96T*5: 5 vials, 10 mL	2-8°C(shading light)
Stop Solution	96T/48T/24T: 1 vial, 10 mL 96T*5: 5 vials, 10 mL	2-8°C
Plate Sealer	96T/48T/24T: 5 pieces 96T*5: 25 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 450 nm 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

Sample collection

(More detailed information please view our website: http://www.elabscience.cn/List– detail-241.html)
Serum: Allow samples to clot for 1 hour 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-Na2 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 10 min 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 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

Recommended reagents for sample preparation: 10×EDTA Anticoagulant (Cat No. E-EL-SR003), PMSF Protease Inhibitor (Cat No. E-EL-SR002), 0.25% Trypsin Solution (Cat No. EP-CM-L0043).

Note

■ Note for kit

1) For research use only. Not for use in diagnostic procedures.
2) 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.
3) 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 and store according to the conditions suggested in the above table.
4) Do not reuse the reconstituted standard, biotinylated detection Ab working solution, 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.
5) The microplate reader should be able to be installed with a filter that can detect the wave length at 450±2 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.
6) Do not mix or substitute reagents with those from other lots or sources.
7) Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.
8) The kit should not be used beyond the expiration date on the kit label.

■ 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) If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
5) If a lysis buffer is used to prepare tissue homogenates or cell lysates, there is a possibility of causing a deviation due to the introduced chemical substance.
6) Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

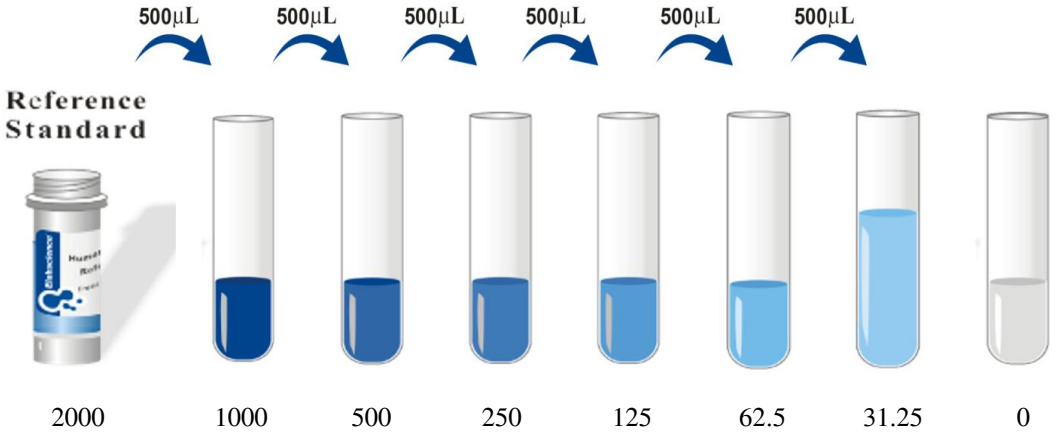
Dilution Method

Please predict the concentration range of samples in advance, and determine the dilution ratio through preliminary experiments or technical support recommendations.

If your test sample needs dilution, please refer to the dilution method as follows:
For 100 fold dilution: One-step dilution. Add 5 μL sample to 495 μL sample diluent to yield 100 fold dilution.
For 1000 fold dilution: Two-step dilution. Add 5 μL sample to 95 μL sample diluent to yield 20 fold dilution, then add 5 μL 20 fold diluted sample to 245 μL sample diluent, after this, the neat sample has been diluted at 1000 fold successfully.
For 100000 fold dilution: Three-step dilution. Add 5 μL sample to 195 μL sample diluent to yield 40 fold dilution, then add 5 μL 40 fold diluted sample to 245 μL sample diluent to yield 50 fold dilution, and finally add 5 μL 2000 fold diluted sample to 245 μL sample diluent, after this, the neat sample has been diluted at 100000 fold successfully.

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 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL 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 2000 pg/mL(or add 1 mL 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: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/mL.
Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 2000 pg/mL working solution to the first tube and mix up to produce a 1000 pg/mL working solution. Pipette 500uL 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. Gradient diluted standard working solution should be prepared just before use.



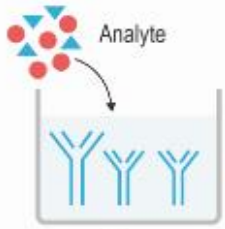
4. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated Biotinylated Detection Ab at 800×g for 1 min, then dilute the 100× Concentrated Biotinylated Detection Ab to 1× working solution with Biotinylated Detection Ab Diluent(Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent= 1: 99).The working solution should be prepared just before use.
5. **Concentrated HRP Conjugate working solution:** HRP Conjugate is HRP conjugated avidin.Calculate the required amount before the experiment (100 μL/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).The working solution should be prepared just before use.

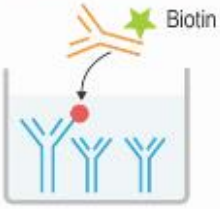
Assay procedure

1. Determine wells for **diluted standard, blank** and **sample**. Add 100 μL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate. It is recommended to determine the dilution ratio of samples through preliminary experiments or technical support recommendations). 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°C.
3. Decant the solution from each well, add 350 μL of **wash buffer** to each well. Soak for 1 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 100 μL of **HRP Conjugate working solution** to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C.
5. Decant the solution from each well, repeat the wash process for 5 times as conducted in step 3.
6. Add 90 μL of **Substrate Reagent** to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement.
7. Add 50 μL 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.

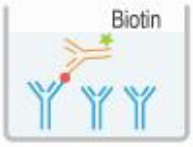
Assay Procedure Summary



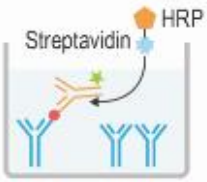
1. Add 100μL standard or sample to the wells. Incubate for 90 min at 37°C



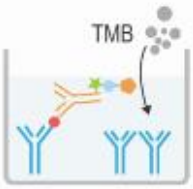
2. Discard the liquid, immediately add 100μL Biotinylated Detection Ab working solution to each well. Incubate for 60 min at 37°C



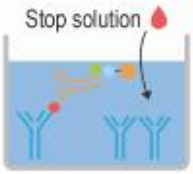
3. Aspirate and wash the plate for 3 times



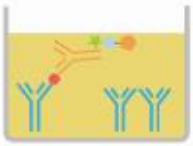
4. Add 100μL HRP conjugate working solution. Incubate for 30 min at 37°C. Aspirate and wash the plate for 5 times



5. Add 90μL Substrate Reagent. Incubate for 15 min at 37°C



6. Add 50μL Stop Solution



7. Read the plate at 450nm immediately. Calculation of the results

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 axis, 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.

Technical resources

More detailed ELISA experiment guidelines and routine problem analysis can be obtained through wechat QR code at the lower left.

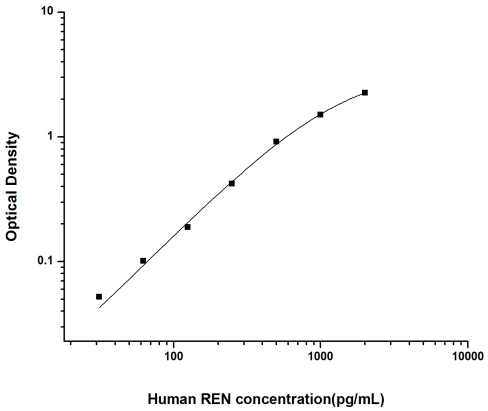
If you have any technical problems, please feel free to contact our technical support (it is recommended to take pictures and save the experimental data in time. Keep the used plate and remaining reagents).

Guidelines for ELISA

Wechat of technical support

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.

pg/mL	OD	Corrected OD	Standard Curve
2000	2.315	2.262	
1000	1.552	1.499	
500	0.966	0.913	
250	0.473	0.42	
125	0.242	0.189	
62.5	0.154	0.101	
31.25	0.105	0.052	
0	0.053	-	

Performance

■ Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level Human REN were tested 20 times on one plate, respectively.
Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level Human REN were tested on 3 different plates, 20 replicates in each plate, respectively.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean(pg/mL)	108.90	258.70	836.60	98.90	234.70	771.40
Standard deviation	5.90	11.90	36.80	6.40	10.80	28.50
C V (%)	5.42	4.60	4.40	6.47	4.60	3.69

■ Recovery

The recovery of Human REN 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)	86-98	92
EDTA plasma (n=8)	84-98	90
Cell culture media (n=8)	83-96	90

■ Linearity

Samples were spiked with high concentrations of Human REN 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 (%)	98-111	96-107	98-110
	Average (%)	104	101	104
1:4	Range (%)	94-106	86-98	97-114
	Average (%)	100	93	104
1:8	Range (%)	99-112	82-95	93-107
	Average (%)	106	88	100
1:16	Range (%)	98-113	86-101	93-106
	Average (%)	106	93	98

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.