Elabscience®

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（本试剂盒仅供体外研究使用，不用于临床诊断！）

大鼠/鸡皮质酮(Corticosterone)使用说明书

Rat/Chicken CORT (Corticosterone) ELISA Kit

产品货号：E-EL-0160c

96T

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

联系时请提供产品货号(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大鼠和鸡血清、血浆及尿液中 CORT 浓度，其余样本类型检测请咨询技术支持。

灵敏度、检测范围、特异性和重复性

●灵敏度：0.24ng/mL

●检测范围：0.39-25ng/mL

●特异性：可检测大鼠和鸡体内的 CORT，且与其它结构类似物无明显交叉反应。

●重复性：板内，板间变异系数均<10%。

背景介绍

皮质酮也称为肾上腺酮，它是由肾上腺外层或皮质分泌的一种类固醇类激素。作为一种糖皮质激素，皮质酮通过肝脏协助调节氨基酸转换为碳水化合物和糖原，刺激组织内糖原的形成。皮质酮在结构上跟其他糖皮质激素如皮质醇和可的松相似，但效力相对较弱。皮质酮由垂体的促肾上腺皮质激素刺激反应产生，在一些非人类的特定物种中，皮质酮是肾上腺分泌的主要的糖皮质激素，它是另一种肾上腺皮质类固醇类物质醛固酮在合成中的前体物质。体内皮质酮含量的增加与焦虑是否得到缓解密切关系，依据皮质酮的注射时间和产生焦虑的时间差，皮质酮可以正向或反向调节焦虑。

检测原理

本试剂盒采用竞争 ELISA 法。将 CORT 抗原包被于酶标板上，实验时样品或标准品中的 CORT 与包被的 CORT 竞争生物素标记的抗 CORT 单抗上的结合位点，游离的成分被洗去。加入辣根过氧化物酶标记的亲和素，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。在酶标仪 450nm 波长处测 OD 值，CORT 浓度与 OD_{450} 值之间呈反比，通过绘制标准曲线计算出样品中 CORT 的浓度。

试剂盒组成及保存

未拆封的试剂盒可在 4℃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	保存条件
ELISA 酶标板 Micro ELISA Plate	8 孔×12 条	-20℃，可存放 6 个月
冻干标准品 Reference Standard	2 支	
浓缩生物素化抗体(100×) Concentrated Biotinylated Detection Ab(100×)	1 支 120μL	
浓缩 HRP 酶结合物(100×) Concentrated HRP Conjugate(100×)	1 支 120μL	-20℃(避光), 可存放 6 个月
标准品&样品稀释 Reference Standard & Sample Diluent	1 瓶 20mL	4℃,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 Concentrated Wash Buffer	1 瓶 30mL	
底物溶液(TMB) Substrate Reagent	1 瓶 10mL	4℃(避光)
反应终止液 Stop Solution	1 瓶 10mL	4℃
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

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。未用完的没有稀释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。
5. 不同批号的试剂盒组份不能混用(洗涤液和反应终止液除外)。
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)

1. 血清：全血样品于室温放置2小时或4℃过夜后于1000×g离心20分钟，取上清即可检测，收集血液的试管应为一次性的无内毒素试管。
2. 血浆：抗凝剂推荐使用EDTA-Na_2，样品采集后30分钟内于1000×g离心15分钟，取上清即可检测。避免使用溶血，高血脂样品。
3. 尿液：用无菌管收集尿液于1000 x g离心15分钟取上清，取上清即可检测，或分装后于-20°C或-80°C保存，避免反复冻融。

样品注意事项

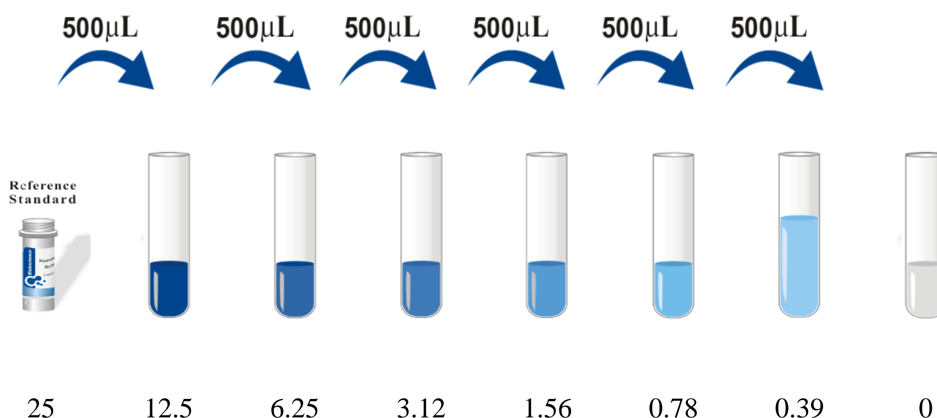
1. 样品收集后若在1周内进行检测的可保存于4℃，若不能及时检测，请按一次使用量分装，冻存于-20℃(1个月内检测)，或-80℃(3个月内检测)，避免反复冻融。
2. 试剂盒检测范围不等同于样本的浓度范围，如果您的样品中检测物浓度高于标准品最高值，请根据实际情况，做适当倍数稀释(建议查阅文献后先做预实验，以确定稀释倍数)。
3. 大鼠来源样本：血清、血浆建议稀释10倍检测。
4. 鸡来源样本：血清建议稀释50倍进行检测，血浆建议稀释20倍进行检测。

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。提前15分钟打开酶标仪预热。

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，配成25ng/mL的标准品工作液。然后根据需要进行倍比稀释。建议配制成以下浓度：25、12.5、6.25、3.12、1.56、0.78、0.39、0ng/mL。倍比稀释方法：取7支EP管，每管中加入500uL**标准品&样品稀释液**，从25ng/mL的标准品工作液中吸取500uL到其中一支EP管中混匀配成12.5ng/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50μL/孔计算)，实际配制时应多配制100-200μL。使用前15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。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，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。当日使用。

操作步骤(操作一览表见第 10 页)

1. 将**标准品工作液**依次加入到前两列孔中，每个浓度的工作液并列加两孔，每孔 50uL。待测样品加入到其他孔,每孔 50μL(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)。立即每孔加入配好的**生物素化抗体工作液** 50μL。给酶标板覆膜，37℃孵育 45 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，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。
3. 每孔加**酶结合物工作液** 100μL，加上覆膜，37℃温育 30 分钟。
4. 弃去孔内液体，甩干，洗板 5 次，方法同步骤 2。
5. 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，即可终止。
6. 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。
7. 立即用酶标仪在 450nm 波长测量各孔的光密度(OD 值)。

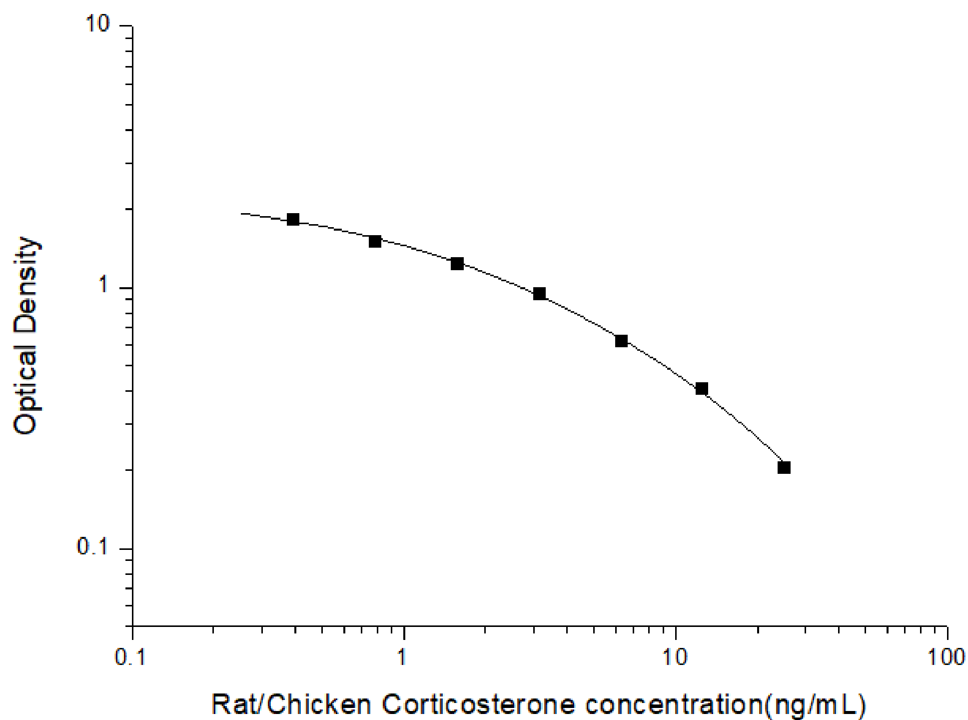
结果判断

1. 计算每组复孔的平均OD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。若样品OD值低于标准曲线下限，应适当稀释后重测。

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。

Concentration(ng/mL)	25	12.5	6.25	3.12	1.56	0.78	0.39	0
OD	0.206	0.410	0.623	0.957	1.249	1.509	1.832	2.352



样本测值

样本类型	不同种属样本检测浓度变化范围(ng/mL)	
	大鼠	鸡
血清(n=10)	75.5-120	51-130
血浆(EDTA)(n=10)	33.42-98.74	48-170
尿液(n=5)	0.9-14.7	-

精密度

板内精密度:大鼠低浓度样本,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:大鼠低浓度样本,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(ng/mL)	30.38	101.43	206.56	31.45	102.78	208.75
标准差	2.66	7.77	14.56	2.91	8.82	18.02
变异系数(%)	8.77	7.66	7.05	9.24	8.58	8.63

回收率

分别往 5 个大鼠来源样本中添加已知浓度的 CORT，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=5)	81-90	85
血浆(EDTA)(n=5)	86-97	89
尿液(n=5)	85-102	96

线性

分别往5个大鼠来源样本中添加已知浓度的CORT，做回收实验，得出回收率范围及平均回收率。将5个样本分别稀释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)	尿液(n=5)
1:2	回收率范围(%)	80-90	88-101	85-106
	平均回收率(%)	85	96	94
1:4	回收率范围(%)	82-94	87-94	80-101
	平均回收率(%)	88	91	92
1:8	回收率范围(%)	84-100	82-89	83-96
	平均回收率(%)	93	86	88
1:16	回收率范围(%)	87-105	89-104	84-99
	平均回收率(%)	91	99	89

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溶解
	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 试剂盒保存不当	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 50μL,立即加入 50uL 生物素化抗体工作液，37°C孵育 45 分钟

2. 洗涤 3 次

3. 加入 100μL 酶结合物工作液， 37°C孵育 30 分钟

4. 洗涤 5 次

5. 加入 90μL 底物溶液， 37°C孵育 15 分钟左右

6. 加入 50μL 终止液，立即在 450nm 波长处测量 OD 值

7. 结果计算

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. 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密切相关，请务必准备充足的待测样品。
3. 为了达到好的实验结果，请只使用 Elabscience 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。
4. 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。
5. 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。
6. 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。
7. 试剂盒有效期：6 个月。

Rat/Chicken CORT(Corticosterone) ELISA Kit

Catalog No: E-EL-0160c

96T

Intended use

This ELISA kit applies to the in vitro quantitative determination of Rat, Chicken CORT concentrations in serum, plasma and urine samples. Please contact tech-support for other sample type detection.

Specification

●Sensitivity: 0.24ng/mL.

●Detection Range: 0.39-25ng/mL

●Specificity: This kit recognizes Rat and Chicken CORT in samples. No significant cross-reactivity or interference between CORT and analogues was observed.

●Repeatability: Coefficient of variation is <10%.

Background

Corticosterone, also known as 17-deoxycortisol, it is a steroid hormone secreted by the outer layer, or cortex, of the adrenal gland. Classed as a glucocorticoid, corticosterone helps regulate the conversion of amino acids into carbohydrates and glycogen by the liver, and helps stimulate glycogen formation in the tissues **[1]**. Corticosterone is similar in structure, although somewhat less potent, than the other glucocorticoids cortisol and cortisone. It is produced in response to stimulation by the pituitary substance adrenocorticotropic hormone (ACTH). In some species, but not in humans, corticosterone is the predominant glucocorticoid secreted by the adrenal. It is a precursor in the synthesis of aldosterone, another adrenal cortical steroid. The increase in corticosterone is linked to anxiety relief. This finding depends on the time at which the administration of corticosterone took place as compared to when the fear conditioning took place, corticosterone can either facilitate or interrupt conditioned fear **[2]**.

1. "Corticosterone." The Columbia Encyclopedia, 6th ed. Retrieved October 24, 2018 from Encyclopedia.com: https://www.encyclopedia.com/reference/encyclopedias-almanacs-transcripts-and-maps/corticosterone
2. Albrecht, A., Çalışkan, G., Oitzl, M. S., Heinemann, U., & Stork, O. (2013). Long-lasting increase of corticosterone after fear memory reactivation: anxiolytic effects and network activity modulation in the ventral hippocampus. *Neuropsychopharmacology*, *38*(3), 386.

Test principle

This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with CORT. During the reaction, CORT in the sample or standard competes with a fixed amount of CORT on the solid phase supporter for sites on the Biotinylated Detection Ab specific to CORT. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of CORT in the samples is then determined by comparing the OD of the samples to the standard curve.

Kit components & Storage

An unopened kit can be stored at 4℃ for 1 week. If the kit is not used within 1 week, store the items separately according to the following conditions since the kit is received.

<table>
<tr><th>Item</th><th>Specifications</th><th>Storage</th></tr>
<tr><td>Micro ELISA Plate (Dismountable)</td><td>8 wells ×12 strips</td><td rowspan="3">-20℃, 6 months</td></tr>
<tr><td>Reference Standard</td><td>2 vials</td></tr>
<tr><td>Concentrated Biotinylated Detection Ab (100×)</td><td>1 vial, 120 uL</td></tr>
<tr><td>Concentrated HRP Conjugate (100×)</td><td>1 vial, 120 μL</td><td>-20℃(shading light), 6 months</td></tr>
<tr><td>Reference Standard & Sample Diluent</td><td>1 vial, 20 mL</td><td rowspan="4">4℃, 6 months</td></tr>
<tr><td>Biotinylated Detection Ab Diluent</td><td>1 vial, 14 mL</td></tr>
<tr><td>HRP Conjugate Diluent</td><td>1 vial, 14 mL</td></tr>
<tr><td>Concentrated Wash Buffer (25×)</td><td>1 vial, 30 mL</td></tr>
<tr><td>Substrate Reagent</td><td>1 vial, 10 mL</td><td>4℃(shading light)</td></tr>
<tr><td>Stop Solution</td><td>1 vial, 10 mL</td><td>4℃</td></tr>
<tr><td>Plate Sealer</td><td>5 pieces</td><td rowspan="3"></td></tr>
<tr><td>Product Description</td><td>1 copy</td></tr>
<tr><td>Certificate of Analysis</td><td>1 copy</td></tr>
</table>

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.

Other supplies required

Microplate reader with 450 nm wavelength filter

High-precision transfer pipette, EP tubes and disposable pipette tips

37℃ Incubator

Deionized or distilled water

Absorbent paper

Loading slot for Wash Buffer

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 to have a water-like substance, which is normal and will not have any impact on the experimental results.
3. Do not reuse the diluted 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.
5. Do not mix or use components 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

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4℃ before centrifugation for 20 min at 1000×g at 2~8℃. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be non-endotoxin.

Plasma: Collect plasma using EDTA-Na_2 as 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. Hemolysed samples are not suitable for ELISA assay!

Urine: Use a sterile container to collect urine samples. Remove particulates by centrifugation for 15 minutes at 1000×g at 2-8 ℃. Collect the supernatant to carry out the assay.

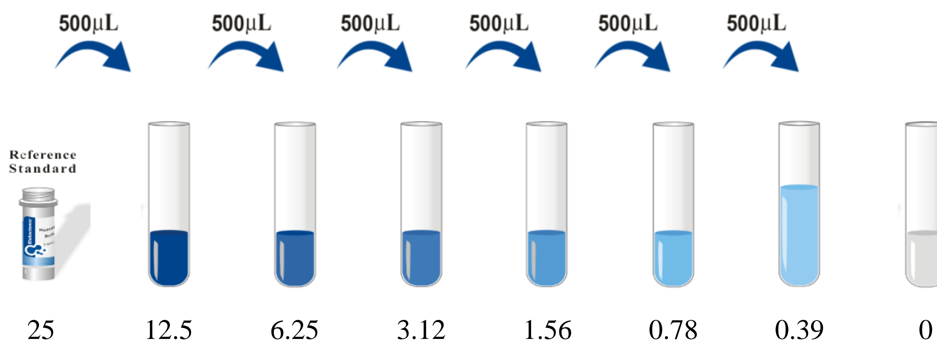
Note for sample:

1. Samples should be assayed within 7 days when stored at 4℃, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤3 months). Avoid repeated freeze-thaw cycles.
2. 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.
3. It is recommended to do the experiment with rat serum and plasma samples diluted at 10 fold.
4. It is recommended to do the experiment with chicken serum samples diluted at 50 fold, plasma samples diluted at 20 fold.

Reagent preparation

1. Bring all reagents to room temperature (18~25℃) before use. Preheat microplate reader for 15 min before OD measurement.
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 1min. Add 1.0mL of Reference Standard &Sample Diluent, let it stand for 10min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 25ng/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 25、12.5、6.25、3.12、1.56、0.78、0.39、0ng/mL.

   Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 25ng/mL working solution to the first tube and mix up to produce a 12.5ng/mL working solution. Pipette 500uL of the solution from the former tube to 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.



4. **Biotinylated Detection Ab working solution:** Calculate the required amount before the experiment (50μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent.
5. **Concentrated HRP Conjugate working solution:** Calculate the required amount before the experiment (100μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100×Concentrated HRP Conjugate to 1×working solution with Concentrated HRP Conjugate Diluent.

Assay procedure (A brief assay procedure is on the 21th page)

1. Add the **Standard working solution** to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side(50 uL for each well). Add the samples to the other wells(50 uL for each well). Immediately add 50 μL of **Biotinylated Detection Ab working solution** to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 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. Aspirate or decant the solution from each well， add 350 uL 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.
3. Add 100 μL of **HRP Conjugate working solution** to each well. Cover with the Plate sealer. Incubate for 30 min at 37 ℃.
4. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 2.
5. Add 90 μL of **Substrate Reagent** to each well. Cover with a new plate 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.
6. 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.
7. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Calculation of results

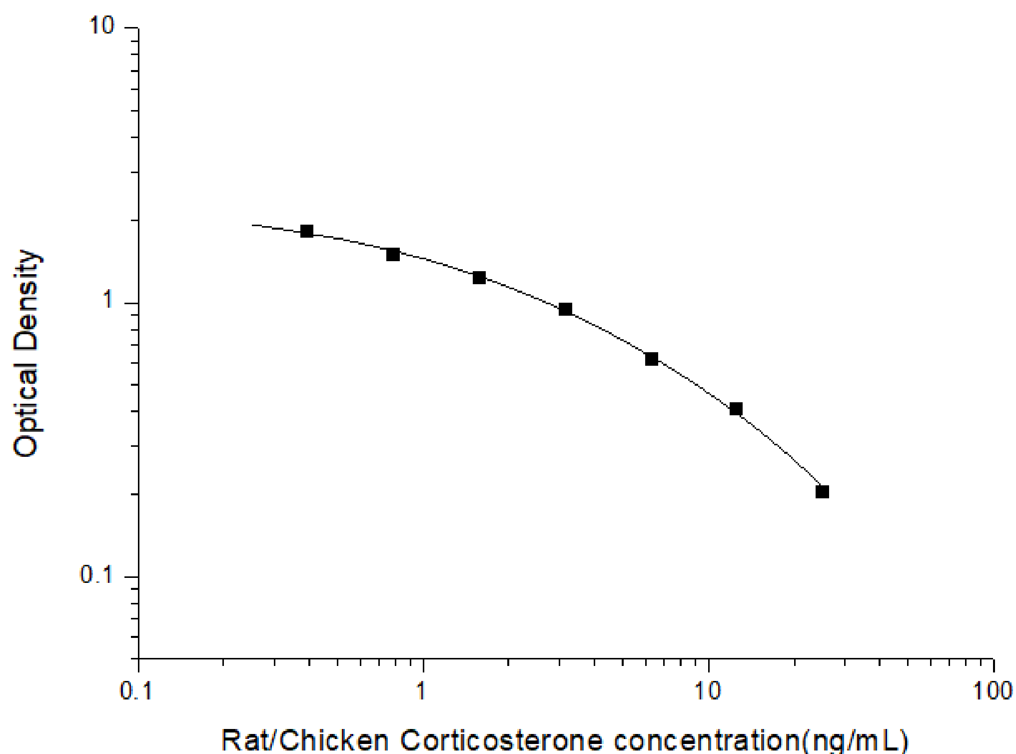
Average the duplicate readings for each standard and samples. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample is under the lowest 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(ng/mL)	25	12.5	6.25	3.12	1.56	0.78	0.39	0
OD	0.206	0.410	0.623	0.957	1.249	1.509	1.832	2.352



Reference values

Samples from rat and chicken were evaluated for the presence of CORT in this assay.

Sample type	Reference range of CORT in different species(ng/mL)	
	Rat	Chicken
Serum(n=10)	75.5-120	51-130
Plasma(EDTA)(n=10)	33.42-98.74	48-170
Urine(n=5)	0.9-14.7	-

Precision

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

Inter-assay Precision (Precision between assays): 3 rat samples with low, mid range and high level CORT 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(ng/mL)	30.38	101.43	206.56	31.45	102.78	208.75
Standard deviation	2.66	7.77	14.56	2.91	8.82	18.02
CV (%)	8.77	7.66	7.05	9.24	8.58	8.63

Recovery

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

Sample Type	Range (%)	Average Recovery (%)
Serum(n=5)	81-90	85
EDTA plasma(n=5)	86-97	89
Urine(n=5)	85-102	96

Linearity

Rat samples were spiked with high concentrations of CORT 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)	urine (n=5)
1:2	Range (%)	80-90	88-101	85-106
	Average (%)	85	96	94
1:4	Range (%)	82-94	87-94	80-101
	Average (%)	88	91	92
1:8	Range (%)	84-100	82-89	83-96
	Average (%)	93	86	88
1:16	Range (%)	87-105	89-104	84-99
	Average (%)	91	99	89

Troubleshooting

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 onthe 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 50 μL standard or sample to each well. Immediately add 50 μL Biotinylated Detection Ab to each well. Incubate for 45 min at 37℃

2. Aspirate and wash 3 times

3. Add 100 μL HRP Conjugate to each well. Incubate for 30 min at 37℃

4. Aspirate and wash 5 times

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

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

7. 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. The final experimental results will be closely related to the validity of products, operational skills of the operators and the experimental environments. Please make sure that sufficient samples are available.
3. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions!
4. 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.
5. 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.
6. 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 equipments, and so on. Intra-assay variance among kits from different batches might arise from the above reasons, too.
7. Valid period: 6 months.

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