（本试剂盒仅供体外研究使用，不用于临床诊断!）

前列腺素E2(PGE2)酶联免疫吸附测定试剂盒
使用说明书

PGE2(Prostaglandin E2) ELISA Kit
产品货号：E-EL-0034c
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

具体保质期请见试剂盒外包装标签。

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血清、血浆或其他相关生物液体中PGE2浓度。

灵敏度、检测范围、特异性和重复性

●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 PGE2，且与其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

本试剂盒采用竞争ELISA法。用PGE2抗原包被于酶标板上，实验时样品或标准品中的PGE2与包被的PGE2竞争生物素标记的抗PGE2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。用酶标仪在450nm波长处测OD值， PGE2浓度与OD450值之间呈反比，通过绘制标准曲线计算出样品中PGE2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，请拆开试剂盒按照下表中的条件分别保存各组分。

中文名称	英文名称	规格	保存条件
ELISA酶标板(可拆卸)	Micro ELISA Plate(Dismountable)	8 孔×12 条	-20°C，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	1支 120 μL	
浓缩HRP酶结合物(100×)	Concentrated HRP Conjugate	1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	1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	1瓶 14 mL	
酶结合物稀释液	HRP Conjugate Diluent	1瓶 14 mL	
浓缩洗涤液（25×）	Concentrated Wash Buffer (25×)	1瓶 30 mL	
底物溶液(TMB)	Substrate Reagent	1瓶 10 mL	2-8°C(避光)
反应终止液	Stop Solution	1瓶 10 mL	2-8°C
封板覆膜	Plate Sealer	5 张	
产品说明书	Product Description	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°C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带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

(具体处理方法参考官网：http://www.elabscience.cn)

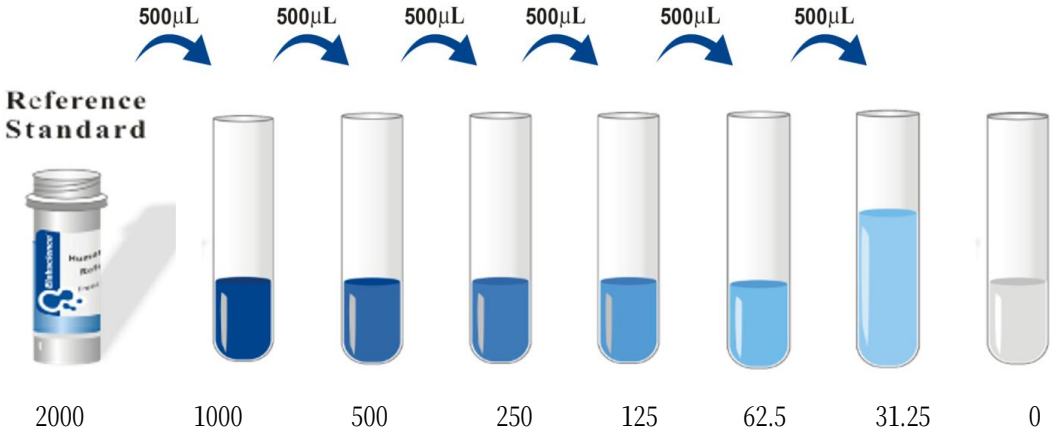
1. 血清：全血样品于室温放置2小时或2-8°C过夜后于1000×g离心20分钟，取上清即可检测，收集血液的试管应为一次性的无内毒素试管。
2. 血浆：抗凝剂推荐使用EDTA钠盐，样品采集后30分钟内于1000×g离心15分钟，取上清即可检测。避免使用溶血，高血脂样品。
3. 组织匀浆：用预冷的PBS (0.01 M,pH=7.4)冲洗组织，去除残留血液，称重后将组织剪碎。将剪碎的组织与对应体积的PBS(一般按1:9的重量体积比，比如1g的组织样品对应9 mL的PBS，具体体积可根据实验需要适当调整，并做好记录。推荐在PBS中加入蛋白酶抑制剂)加入玻璃匀浆器中，在冰上充分研磨。为了进一步裂解组织细胞，可以对匀浆液进行超声破碎或反复冻融。最后将匀浆液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 1×10^6 个细胞中加入150-200 μL PBS重悬并通过反复冻融使细胞破碎(若含量很低可减少PBS的体积)。将提取液于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： 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. 样品收集后若在1周内进行检测的可保存于2-8°C，若不能及时检测，请按一次使用量分装，冻存于-20°C(1个月内检测)，或-80°C(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. 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。
4. 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。
5. 某些重组蛋白可能与试剂盒中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. 请提前20分钟从冰箱中取出试剂盒，平衡至室温。读数前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°C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1.0 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，配成2000pg/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：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500μL标准品&样品稀释液,从2000pg/mL的标准品工作液中吸取500μL到其中一支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。



4. 生物素化抗体工作液：实验前计算实验所需用量(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(第10页中附有简版操作概要)

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50μL。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)。

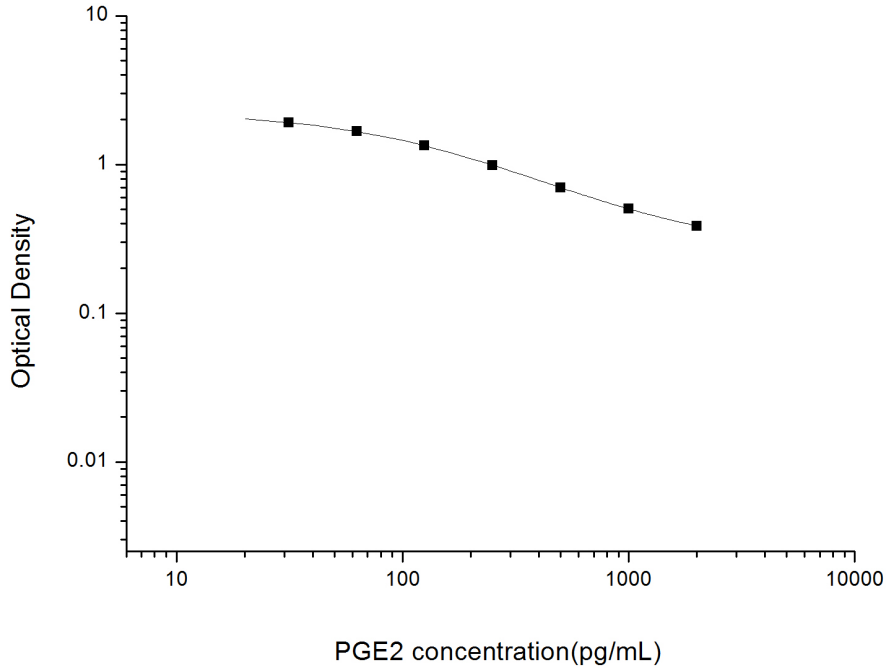
结果判断

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。
2. 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

由于实验操作条件的不同（如操作者、移液技术、洗板技术和稳定条件等），标准曲线的OD值会有所差异。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(pg/mL)	2000	1000	500	250	125	62.5	31.25	0
OD	0.387	0.503	0.699	0.988	1.336	1.668	1.919	2.265



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。

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean(pg/mL)	101.30	236.80	779.30	96.00	259.90	728.60
Standard deviation	6.60	11.60	42.10	4.90	13.80	31.30
C V (%)	6.52	4.90	5.40	5.10	5.31	4.30

回收率

分别往5个不同样本中添加已知浓度的目标蛋白，做回收实验，得出回收率范围和平均回收率。

Sample Type	Range (%)	Average Recovery (%)
Serum (n=5)	92-109	99
EDTA plasma (n=5)	87-99	94

线性

分别往5个样本中添加已知浓度的目标蛋白，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。

		Serum (n=5)	EDTA plasma(n=5)	Cell culture media(n=5)
1:2	Range (%)	95-110	91-104	89-101
	Average (%)	103	98	96
1:4	Range (%)	85-95	89-101	101-117
	Average (%)	90	95	107
1:8	Range (%)	87-100	92-104	96-111
	Average (%)	92	97	102
1:16	Range (%)	88-104	93-105	99-114
	Average (%)	95	98	106

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50 μL,立即加入50 μL生物素化抗体工作液，37℃孵育45分钟
2. 洗涤3次
3. 加入100 μL酶结合物工作液， 37℃孵育30分钟
4. 洗涤5次
5. 加入90 μL底物溶液， 37℃孵育15分钟左右
6. 加入50 μL终止液，立即在450 nm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。

PGE2(Prostaglandin E2) ELISA Kit

Synonyms: PG-E2, Dinoprostone

Catalog No : E-EL-0034c
96T

Intended use
This ELISA kit applies to the in vitro quantitative determination of PGE2 concentrations in serum, plasma and other biological fluids.

Specification

●Sensitivity: 18.75 pg/mL
●Detection Range: 31.25-2000 pg/mL

●Specificity: This kit recognizes PGE2 in samples. No significant cross-reactivity or interference between PGE2 and analogues was observed.

●Repeatability: Coefficient of variation is < 10%.

Test principle
This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated wit PGE2. During the reaction, PGE2 in the sample or standard competes with a fixed amount of PGE2 on the solid phase supporter for sites on the Biotinylated Detection Ab specific to PGE2. 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 PGE2 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 2-8℃ for 1 week. If the kit is not 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)	8 wells ×12 strips	-20℃, 6 months
Reference Standard	2 vials	
Concentrated Biotinylated Detection Ab (100×)	1 vial, 120 μL	
Concentrated HRP Conjugate (100×)	1 vial, 120 μL	-20°C(shading light), 6 months
Reference Standard & Sample Diluent	1 vial, 20 mL	4°C, 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	4°C(shading light)
Stop Solution	1 vial, 10 mL	4°C
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 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 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 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 have a 450(±10 nm) filter installed and a detector that can detect the wavelength. 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 standards, in between sample additions, and in between reagent additions. Also, use separate reservoirs for each reagent.

Sample collection

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. Blood collection tubes should be disposable and be non-endotoxin.

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. Hemolysed samples are not suitable for ELISA assay!

Tissue homogenates: It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolysed 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 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 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. Avoid repeated freeze-thaw cycles.

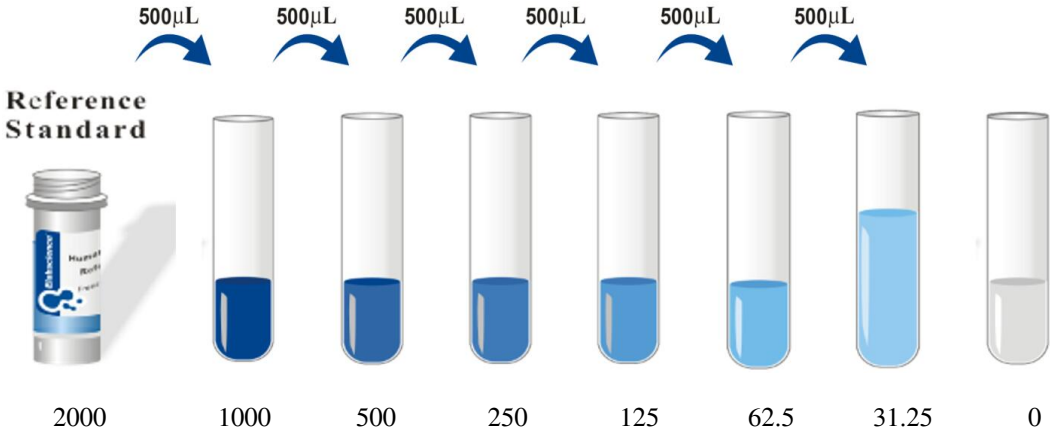
Cell culture supernatant or other biological fluids: Centrifuge samples for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

Note for sample:

1. 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.
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. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
4. 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.
5. Some recombinant protein may not be detected due to a mismatching with the detection antibody.

Reagent preparation

1. Bring all reagents to room temperature (18~25℃) before use. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.
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. 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 stock 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 these steps. 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 (50 μL/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 (100 μL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100× Concentrated HRP Conjugate to 1× working solution with Concentrated HRP Conjugate Diluent.

Assay procedure (A brief assay procedure is on the 20th 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 50μL 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°C.
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°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 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.

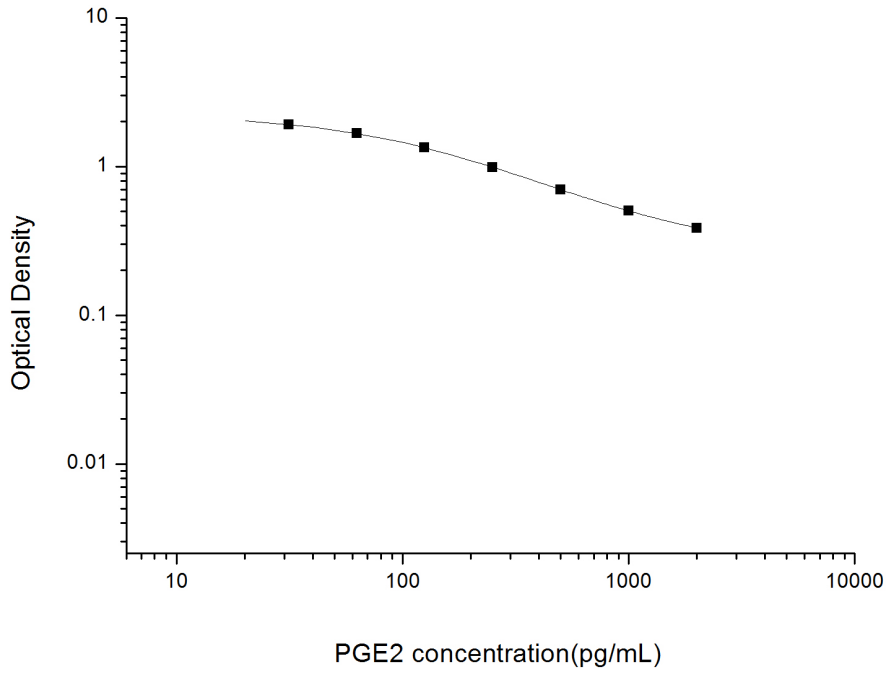
Calculation of results

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(pg/mL)	2000	1000	500	250	125	62.5	31.25	0
OD	0.387	0.503	0.699	0.988	1.336	1.668	1.919	2.265



Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level PGE2 were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level PGE2 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)	101.30	236.80	779.30	96.00	259.90	728.60
Standard deviation	6.60	11.60	42.10	4.90	13.80	31.30
C V (%)	6.52	4.90	5.40	5.10	5.31	4.30

Recovery

The recovery of PGE2 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=5)	92-109	99
EDTA plasma (n=5)	87-99	94
Cell culture media (n=5)	86-96	91

Linearity

Samples were spiked with high concentrations of PGE2 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 (%)	95-110	91-104	89-101
	Average (%)	103	98	96
1:4	Range (%)	85-95	89-101	101-117
	Average (%)	90	95	107
1:8	Range (%)	87-100	92-104	96-111
	Average (%)	92	97	102
1:16	Range (%)	88-104	93-105	99-114
	Average (%)	95	98	106

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 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 50μL standard or sample to each well. Immediately add 50μL Biotinylated Detection Ab to each well. Incubate for 45 min at 37℃.
2. Aspirate and wash 3 times.
3. Add 100μL HRP Conjugate to each well. Incubate for 30 min at 37℃.
4. Aspirate and wash 5 times.
5. Add 90μL Substrate Reagent. Incubate 15 min at 37℃.
6. Add 50μL 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.