Enzyme-linked Immunosorbent Assay Kit

For Sheep peroxisome proliferators-activated receptors γ (PPARγ)

Instruction manual

FOR IN VITRO AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

3th Edition (Revised in September, 2015)

- **For the quantitative in vitro determination of Sheep peroxisome proliferators-activated receptors γ concentrations in serum - plasma - tissue homogenates - other biological fluids**
- **Storage: 2-8℃ (Use frequently)**
- **Validity：six months (-20℃)。**

This package insert must be read in its entirety before using this product.

[INTENDED USE AND TEST PRINCIPLE]

This PPAR γ ELISA kit is intended Laboratory for Research use only and is not for use in diagnostic or therapeutic procedures. The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of PPAR γ in the sample, this PPAR γ ELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versus PPAR γ concentration. The concentration of PPAR γ in the samples is then determined by comparing the O.D. of the samples to the standard curve.

[SAMPLE COLLECTION AND STORAGES]

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4℃ before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 ℃ within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 ℃ or -80 ℃ for later use. Avoid repeated freeze/thaw cycles.

Tissue homogenates - For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate.

Cell culture supernates and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20℃ or -80 ℃ for later use. Avoid repeated freeze/thaw cycles.

Note: The samples shoule be centrifugated dequately and no hemolysis or granule was allowed.

[MATERIALS REQUIRED BUT NOT SUPPLIED]

1. 37 ℃ incubator
2. Standard microplate reader capable of measuring absorbance at 450 nm
3. Precision pipettes, disposable pipette tips and Absorbent paper
4. Distilled or deionized water

[REAGENTS PROVIDED]

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

Name	96 determinations	48 determinations
MICROTITER PLATE	8*12strips	8*6strips
STANDARD（6 vial）	0.3ml/vial	0.3ml/vial
SAMPLE DILUENT	6.0ml	3.0ml
ENZYME CONJUGATE	10.0ml	5.0ml
WASH SOLUTION	25ml	15ml
SUBSTRATE A	6.0ml	3.0ml
SUBSTRATE B	6.0ml	3.0ml
STOP SOLUTION	6.0ml	3.0ml
Closure plate membrane	2	2
User manual	1	1
Sealed bags	1	1
PLATE	1	1
RUBBER	2	2

Note:

1. Standard concentration was followed by: 80, 40, 20, 10, 5, 2.5 pg/ml.
2. If samples generate values higher than the highest standard, please dilute the samples with Sample Diluent and repeat the assay.

[PRECAUTIONS]

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from Rat blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate viruses.
10. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
11. Substrate Solution is easily contaminated. If bluish prior to use, do not use.
12. Substrate B contain 20% acetone, keep this reagent away from sources of heat or flame.
13. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C).

[REAGENT PREPARATION AND STORAGE]

Wash Solution (1X) - Dilute 1 volume of Wash solution (20X) with 19 volumes of deionized or distilled water. Wash Solution is stable for 1 month at 2-8°C.

[ASSAY PROCEDURE]

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microtiter plate.
2. Add 50µl of Standard or Sample to the appropriate wells. Blank well doesn't add anyting.

3. Add 100µl of Enzymeconjugate to standard wells and sample wells except the blank well, cover with an adhesive strip and incubate for 60 minutes at 37°C.

4. Wash the Microtiter Plate 4 times.

Manual Washing - Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Solution (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure for a total of four times. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

Automated Washing - Aspirate all wells, then wash plates four times using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350µL/well/wash. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.

5. Add Substrate A 50µl and Substrate B 50µl to each well. Gently mix and incubate for 15 minutes at 37°C. **Protect from light.**

6. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

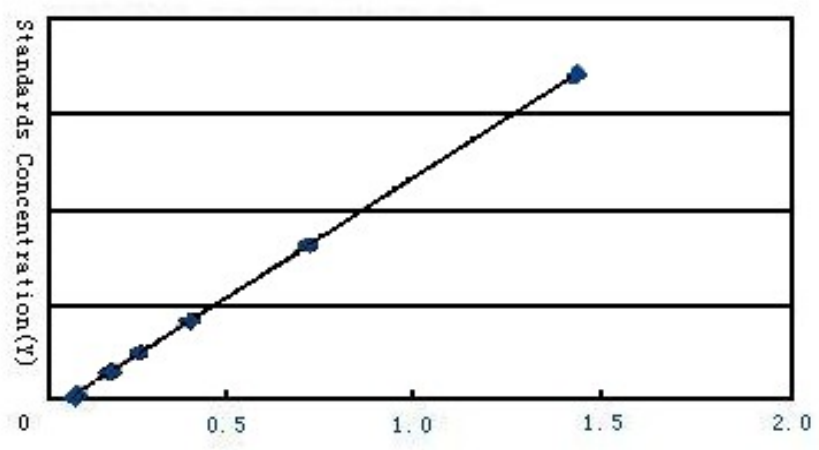
[CALCULATION OF RESULTS]

1. This standard curve is used to determine the amount in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (X) axis versus the corresponding concentration on the horizontal (Y) axis.

2. First, calculate the mean O.D. value for each standard and sample. All O.D. Values are subtracted by the mean value of the balnk well before result interpretation. Construct the standard curve using graph paper or statistical software.

3. To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.

4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.
5. Intra-assay CV(%) is less than 10% and Inter-assay CV(%) is less than 15%.
6. Assay range: 2.5 pg/ml – 80 pg/ml.
7. Sensitivity: The minimum detectable dose of Sheep PPAR γ is typically less than 0.1 pg/ml.
8. Cross-reactivity: This assay recognizes recombinant and natural Sheep PPAR γ . No significant cross-reactivity or interference was observed.
9. Storage: 2-8℃ (Use frequently); six months (-20℃)。
10. Standard curve



FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS! PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

羊过氧化物酶体增殖物激活受体γ（PPARγ）试剂盒

（酶联免疫吸附试验法）

使用说明书

仅供体外研究使用，不用于临床诊断！

第 3 版（2015 年 09 月修订）

- **本试剂盒用于体外定量检测血清、血浆、组织匀浆及相关液体样本中羊过氧化物酶体增殖物激活受体γ（PPARγ）的含量。**
- **有效期：6个月**
- **保存条件：2-8℃**

[实验原理]

试剂盒采用双抗体一步夹心法酶联免疫吸附试验（ELISA）。往预先包被羊过氧化物酶体增殖物激活受体γ（PPARγ）捕获抗体的包被微孔中，依次加入标本、标准品、HRP标记的检测抗体，经过温育并彻底洗涤。用底物TMB显色，TMB在过氧化物酶的催化下转化成蓝色，并在酸的作用下转化成最终的黄色。颜色的深浅和样品中的羊过氧化物酶体增殖物激活受体γ（PPARγ）呈正相关。用酶标仪在450nm 波长下测定吸光度（OD 值），计算样品浓度。

[样本处理及要求]

1. 血清：将收集于血清分离管的全血标本在室温放置2小时或4℃过夜，然后1000×g离心20分钟，取上清即可，或将上清置于-20℃或-80℃保存，但应避免反复冻融。

2. 血浆：用EDTA或肝素作为抗凝剂采集标本，并将标本在采集后的30分钟内于2-8℃ 1000×g离心15分钟，取上清即可检测，或将上清置于-20℃或-80℃保存，但应避免反复冻融。

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（匀浆中裂解的红细胞会影响测量结果），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，于冰上充分研磨。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。最后将匀浆液于5000×g离心5~10分钟，取上清检测。

4. 细胞培养物上清或其它生物标本：请1000×g离心20分钟，取上清即可检测，或将上清置于-20℃或-80℃保存，但应避免反复冻融。

注：标本溶血会影响最后检测结果，因此溶血标本不宜进行此项检测。

[需要而未提供的试剂和器材]

1. 酶标仪（450nm）
2. 高精度加样器及枪头：0.5-10uL、2-20uL、20-200uL、200-1000uL
3. 37℃恒温箱
4. 蒸馏水或去离子水

[试剂盒组成]

名称	96 孔配置	48 孔配置	备注
微孔酶标板	8 孔×12 条	8 孔×6 条	无
标准品	0.3mL*6 管	0.3mL*6 管	无
样本稀释液	6mL	3mL	无
检测抗体-HRP	10mL	5mL	无
20×洗涤缓冲液	25mL	15mL	按说明书进行稀释
底物 A	6mL	3mL	无
底物 B	6mL	3mL	无
终止液	6mL	3mL	无
封板膜	2 张	2 张	无
说明书	1 份	1 份	无
自封袋	1 个	1 个	无
空白稀释板	1 块	1 块	选配
橡胶手套	2 副	2 副	选配

备注：

1. 标准品浓度依次为：80、40、20、10、5、2.5 pg/ml
2. 经过大量正常标本检验，标本的正常浓度值均在试剂盒提供的检测范围内，实验过程中直接取50μL样本上样即可。当有部分样本值超过最大标准品浓度时，可用样本稀释液将标本进行适当稀释后再进行实验。

[注意事项]

1. 严格按照规定的时间和温度进行温育以保证准确结果。所有试剂都必须在使用前达到室温 20-25℃。使用后立即冷藏保存试剂。
2. 洗板不正确可以导致不准确的结果。在加入底物前确保尽量吸干孔内液体。温育过程中不要让微孔干燥掉。
3. 消除板底残留的液体和手指印，否则影响 OD 值。
4. 底物显色液应呈无色或很浅的颜色，已经变蓝的底物液不能使用。
5. 避免试剂和标本的交叉污染以免造成错误结果。
6. 在储存和温育时避免强光直接照射。
7. 平衡至室温后再打开密封袋以防水滴凝聚在冷板条上。
8. 任何反应试剂不能接触漂白溶剂或漂白溶剂所散发的强烈气体。任何漂白成分都会破坏试剂盒中反应试剂的生物活性。
9. 不能使用过期产品。
10. 如果可能传播疾病，所有的样品都应管理好，按照规定的程序处理样品和检测装置。

[试剂准备]

试剂盒从冷藏环境中取出应在室温平衡后方可使用。

20×洗涤缓冲液的稀释：蒸馏水按1：20稀释，即1份20×洗涤缓冲液加19份蒸馏水。

[操作步骤]

1. 从室温平衡 20min 后的铝箔袋中取出所需板条，剩余板条用自封袋密封放回 4℃。
2. 设置标准品孔和样本孔，标准品孔各加不同浓度的标准品 50μL；
3. 样本孔中加入待测样本 50μL；空白孔不加。
4. 除空白孔外，标准品孔和样本孔中每孔加入辣根过氧化物酶（HRP）标记的检测抗体 100μL，用封板膜封住反应孔，37℃水浴锅或恒温箱温育 60min。
5. 弃去液体，吸水纸上拍干，每孔加满洗涤液（350μL），静置 1min，甩去洗涤液，吸水纸上拍干，如此重复洗板 5 次（也可用洗板机洗板）。
6. 每孔加入底物 A、B 各 50μL，37℃避光孵育 15min。
7. 每孔加入终止液 50μL，15min 内，在 450nm 波长处测定各孔的 OD 值。

[实验结果计算]

以所测标准品的OD值为横坐标，标准品的浓度值为纵坐标，在坐标纸上或用相关软件绘制标准曲线，并得到直线回归方程，将样品的OD值代入方程，计算出样品的浓度。

[试剂盒性能]

1. 检测范围：2.5 pg/ml – 80 pg/ml。
2. 灵敏度：最低检测浓度小于 0.1 pg/ml。
3. 特异性：不与其它可溶性结构类似物交叉反应。
4. 重复性：板内变异系数小于 10% ，板间变异系数小于 15% 。

[说明]

1. 由于现有条件及科学技术水平尚不能对所有供货商提供的所有原料进行全面的鉴定与分析，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，请务必准备充足的标本备份。
3. 不同批次的同一产品可能会有少许差别，如：检测限、灵敏度以及显色时间等，请依据试剂盒内说明书进行实验操作，网站电子版说明书仅作参考。
4. 只有全部使用本试剂盒配套试剂才能保证检测效果，不能混用其他制造商的产品。只有严格遵守本试剂盒的实验说明才会得到最佳的检测结果。
5. 本公司只对试剂盒本身负责，不对因使用该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的可能使用量，预留充足的样本。
6. 使用化学裂解液制备的组织匀浆或细胞提取液可能会由于某些化学物质的引入导致 ELISA 实验结果偏差。
7. 若样本为细胞培养上清，因该类样本干扰因素较多，如：细胞状态、细胞数量、采样时间等，所以可能存在检测不出的情况。
8. 某些天然蛋白或重组蛋白，包括原核及真核重组蛋白，可能因为与本产品所使用的检测抗体及捕获抗体不匹配，而不被检测出。