Human Interleukin 27 (IL-27) ELISA Kit

Catalog No. CSB-E08464h

(96T)

- This immunoassay kit allows for the in vitro quantitative determination of **human IL-27** concentrations in **serum, plasma and other biological fluids.**
- **Expiration date** **six** months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

INTRODUCTION

Interleukin-27 (IL-27) is a heterodimeric cytokine belonging to the IL-12 family that is composed of two subunits, Epstein-Barr virus (EBV)-induced gene 3 (EBI3) (also known as IL-27B) and IL27-p28 (known as IL-30). IL-27 is produced by antigen-presenting cells. IL-27 plays an important function in regulating the activity of B and T lymphocytes. The effects of IL-27 are eliciting by its interaction with a specific cell surface receptor complex composed of two proteins known as WSX-1 (TCCR) and gp130. IL-27 binds and signals through a heterodimeric receptor complex consisting of WSX-1 and gp130, both belonging to the cytokine receptor superfamily. WSX-1 is specific for IL-27 and is expressed on resting/naive CD4+ T cells, CD8+ T cells, NK cells, dendritic cells, monocytes, mast cells, and B cells. In contrast, gp130 is ubiquitously expressed by a variety of immune and non-immune cells and functions as a subunit of the receptor complexes for at least seven other cytokines. IL-27 has both pro- and anti-inflammatory properties. In response to infection, IL-27 induces monocytes and mast cells to secrete pro-inflammatory cytokines. It induces naive CD4+ T cells to proliferate and develop Th1 cell responses. IL-27 also promotes effector functions of NK cells and CD8+ T cells. As an anti-inflammatory immunomodulator, IL-27 has been found to have the ability to inhibit Th1 or Th2 responses and restrict the strength and duration of adaptive immune responses.

PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with an antibody specific to IL-27. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for IL-27 and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain IL-27, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of IL-27 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

DETECTION RANGE

1.56 pg/ml-100 pg/ml. The standard curve concentrations used for the ELISA's were 100 pg/ml, 50 pg/ml, 25pg/ml, 12.5 pg/ml, 6.25 pg/ml, 3.12 pg/ml, 1.56 pg/ml.

SPECIFICITY

This assay recognizes recombinant and natural human IL-27. No significant cross-reactivity or interference was observed.

SENSITIVITY

The minimum detectable dose of human IL-27 is typically less than 0.39 pg/ml.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	1
Standard	2
Sample Diluent	1 x 20 ml
Biotin-antibody Diluent	1 x 10 ml
HRP-avidin Diluent	1 x 10 ml
Biotin-antibody	1 x 120µl
HRP-avidin	1 x 120µl
Wash Buffer	1 x 20 ml (25×concentrate)
TMB Substrate	1 x 10 ml
Stop Solution	1 x 10 ml

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag. The test kit may be used throughout the expiration date of the kit, provided it is stored as prescribed above. Refer to the package label for the expiration date.

2. Opened test plate should be stored at 2-8°C in the aluminum foil bag with desiccants to minimize exposure to damp air. The kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

1. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 500 ml of Wash Buffer.
2. **Standard** Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the **Standard** with 1.0 ml of **Sample Diluent**. This reconstitution produces a stock solution of 100 pg/ml. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high standard (100 pg/ml). The **Sample Diluent** serves as the zero standard (0 pg/ml). Prepare fresh for each assay. Use within 4 hours and discard after use.

3. **Biotin-antibody** Centrifuge the vial before opening. Dilute to the working concentration using **Biotin-antibody Diluent**(1:100), respectively.
4. **HRP-avidin** Centrifuge the vial before opening. Dilute to the working concentration using **HRP-avidin Diluent**(1:100), respectively.

Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 g. Remove serum and assay immediately or aliquot and store samples at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

- **Plasma** Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate. All the reagents should be added directly to the liquid level in the well. The pipette should avoid contacting the inner wall of the well.

1. Add 100µl of Standard, Blank, or Sample per well. Cover with the adhesive strip. Incubate for 2 hours at 37°C.
2. Remove the liquid of each well, don't wash.
3. Add 100µl of **Biotin-antibody** working solution to each well. Incubate for 1 hour at 37°C. **Biotin-antibody** working solution may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.
4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash: Fill each well with Wash Buffer (200µl) and let it stand for 2 minutes, then remove the liquid by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel. Complete removal of liquid at each step is essential to good performance.

5. Add 100µl of **HRP-avidin** working solution to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 1 hour at 37°C.
6. Repeat the aspiration and wash three times as step 4.
7. Add 90µl of **TMB Substrate** to each well. Incubate for 10-30 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
8. Add 50µl of **Stop Solution** to each well when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Using the professional soft "Curve Exert 1.3" to make a standard curve is recommended, which can be downloaded from our web.

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the

points on the graph. The data may be linearized by plotting the log of the IL-27 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the Standard Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Standard Diluent and repeat the assay.
- Any variation in Standard Diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- Centrifuge vials before opening to collect contents.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless or light blue until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless or light blue to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

人白介素 27(IL-27)酶联免疫分析

试剂盒使用说明书

本试剂盒仅供研究使用

产品编号： CSB-E08464h

检测范围： 1.56 pg/ml - 100 pg/ml

最低检测限： 0.39pg/ml

特异性： 本试剂盒可同时检测天然或重组的人 IL-27，且与其他相关蛋白无交叉反应。

有效期： 6 个月

预期应用： ELISA 法定量测定人血清、血浆、细胞培养上清或其它相关生物液体中 IL-27 含量。

说明

1 试剂盒保存：未开封的试剂盒应储存于 2-8℃；开封后的酶标板应与干燥剂一起储存于铝箔袋中置于 2-8℃保存。仅在此出储存条件下，产品在有效期内可正常使用。
2 浓洗涤液低温保存会有盐析出，稀释时可在水浴中加温助溶。
3 中、英文说明书可能会有不一致之处，请以英文说明书为准。
4 刚开启的酶联板孔中可能会含有少许水样物质，此为正常现象，不会对实验结果造成任何影响。

实验原理

用纯化的抗体包被微孔板，制成固相载体，往包被抗 IL-27 抗体的微孔中依次加入标本或标准品、生物素化的抗 IL-27 抗体、HRP 标记的亲和素，经过彻底洗涤后用底物 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 IL-27 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。

试剂盒组成及试剂配制

1. **酶联板(Assay plate)**：一块（96 孔）。
2. **标准品（Standard）**：2 瓶(冻干品)。
3. **样品稀释液(Sample Diluent)**：1×20ml/瓶。
4. **生物素标记抗体稀释液（Biotin-antibody Diluent）**：1×10ml/瓶。
5. **辣根过氧化物酶标记亲和素稀释液（HRP-avidin Diluent）** 1×10ml/瓶。
6. **生物素标记抗体（Biotin-antibody）**：1×120μl/瓶（1：100）。
7. **辣根过氧化物酶标记亲和素（HRP-avidin）**：1×120μl/瓶（1：100）。
8. **底物溶液（TMB Substrate）**：1×10ml/瓶。
9. **浓洗涤液（Wash Buffer）** 1×20ml/瓶，使用时每瓶用蒸馏水稀释 25 倍。
10. **终止液（Stop Solution）**：1×10ml/瓶。

需要而未提供的试剂和器材

1. 标准规格酶标仪
2. 高速离心机
3. 电热恒温培养箱
4. 干净的试管和 Eppendof 管
5. 系列可调节移液器及吸头，一次检测样品较多时，最好用多通道移液器
6. 蒸馏水，容量瓶等

标本的采集及保存

1. 血清：全血标本请于室温放置 2 小时或 4℃过夜后于 1000g 离心 20 分钟，取上清即可立即检测；或进行分装，并将标本放于-20℃或-80℃保存，但应避免反复冻融。解冻后的样品应再次离心，然后检测。
2. 血浆：可用 EDTA 或肝素作为抗凝剂，标本采集后 30 分钟内于 2 - 8°C 1000 g 离心 15 分钟，取上清即可立即检测；或进行分装，并将标本放于-20℃或-80℃保存，但应避免反复冻融。解冻后的样品应再次离心，然后检测。

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。

标准品的稀释原则：2 瓶，使用前于 6000-10000rpm 离心 30 秒。 每瓶临用前以样品稀释液稀释至 1ml，盖好后静置 10 分钟以上，然后反复颠倒/搓动以助溶解，其浓度为 100pg/ml，做系列倍比稀释后，分别稀释 100 pg/ml, 50 pg/ml, 25pg/ml, 12.5 pg/ml, 6.25 pg/ml, 3.12 pg/ml, 1.56 pg/ml，样品稀释液直接作为标准浓度 0 pg/ml，临用前 15 分钟内配制，用完丢弃，下次检测使用新鲜配置的标准品。

如配制 50 pg/ml 标准品：取 0.5ml（不要少于 0.5ml）100 pg/ml 的上述标准品加入含 0.5ml 样品稀释液的 Eppendorf 管中，混匀即可，其余浓度以此类推。

生物素标记抗体的稀释原则：

打开瓶盖前请离心，收集瓶壁上的溶液。临用前以生物素标记抗体稀释液稀释，稀释前根据预先计算好的每次实验所需的总量配制（每孔 100μl），实际配制时应多配制 0.1-0.2ml。如 10μl 生物素标记抗体加 990μl 生物素标记抗体稀释液的比例配制，轻轻混匀，在使用前一小时内配制。

辣根过氧化物酶标记亲和素的稀释原则：

打开瓶盖前请离心，收集瓶壁上的溶液。临用前以辣根过氧化物酶标记亲和素稀释液稀释，稀释前根据预先计算好的每次实验所需的总量配制（每孔 100μl），实际配制时应多配制 0.1-0.2ml。如 10μl 辣根过氧化物酶标记亲和素加 990μl 辣根过氧化物酶标记亲和素稀释液 的比例配制，轻轻混匀，在使用前一小时内配制。

操作步骤

实验开始前，请提前配置好所有试剂，试剂或样品稀释时，均需混匀，混匀时尽量避免起泡。每次检测都应该做标准曲线。如样品浓度过高时，用样品稀释液进行稀释，以使样品符合试剂盒的检测范围。加样时，枪头应直接对准液面，切勿沿孔壁加样。

1 加样：分别设空白孔、标准孔、待测样品孔。空白孔加样品稀释液 100μl，余孔分别加标准品或待测样品 100μl，注意不要有气泡，加样将样品加于酶标板孔底部，尽量不触及孔壁，轻轻晃动混匀，酶标板加上盖或覆膜，37℃反应 120 分钟。

为保证实验结果有效性，每次实验请使用新的标准品溶液。

2 弃去液体，甩干，不用洗涤。每孔加生物素标记抗体工作液 100μl（取 1μl 生物素标记抗体加 99μl 生物素标记抗体稀释液的比例配制，轻轻混匀，在使用前一小时内配制），37℃,60 分钟。

3 温育 60 分钟后，弃去孔内液体，甩干，洗板 3 次，每次浸泡 1-2 分钟，200μl/每孔，甩干。

4 每孔加辣根过氧化物酶标记亲和素工作液（同生物素标记抗体工作液）100μl，37℃，60 分钟。

5 温育 60 分钟后，弃去孔内液体，甩干，洗板 5 次，每次浸泡 1-2 分钟，200μl/每孔，甩干。

6 依序每孔加底物溶液 90μl，37℃避光显色（20-30 分钟内，此时肉眼可见标准品的前 3-4 孔有明显的梯度蓝色，后 3-4 孔显色不明显，即可终止）。

7 依序每孔加终止溶液 50μl，终止反应（此时蓝色立转黄色）。终止液的加入顺序应尽量与底物液的加入顺序相同。为了保证实验结果的准确性，底物反应时间到后应尽快加入终止液。

8 用酶联仪在 450nm 波长依序测量各孔的光密度（OD 值）。 在加终止液后 15 分钟以内进行检测。

实验备注

1. 用户在初次使用试剂盒时，应将各种试剂管离心数分钟，以便试剂集中到管底。
2. 每次实验留一孔作为空白调零孔，该孔不加任何试剂，只是最后加底物溶液及终止液。测量时先用此孔调 OD 值至零。
3. 为防止样品蒸发，试验时将反应板放于铺有湿布的密闭盒内，酶标板加上盖或覆膜。
4. 未使用完的酶标板或者试剂，请于 2-8℃保存。标准品、生物素标记抗体工作液、辣根过氧化物酶标记亲和素工作液请依据所需的量配置使用。请勿重复使用已稀释过的标准品、生物素标记抗体工作液、或辣根过氧化物酶标记亲和素工作液。
5. 建议检测样品时均设双孔测定，以保证检测结果的准确性。

洗板方法

手工洗板方法：吸去（不可触及板壁）或甩掉酶标板内的液体；在实验台上铺垫几层吸水纸，酶标板朝下用力拍几次；将推荐的洗涤缓冲液至少 0.3ml 注入孔内，浸泡 1-2 分钟。根据需要，重复此过程数次。

自动洗板：如果有自动洗板机，应在熟练使用后再用到正式实验过程中。

计算

请从我们的网站下载专业软件"Curve Exert 1.3"，并根据提示制作标准曲线。以标准物的浓度为横坐标（对数坐标），OD 值为纵坐标（普通坐标），在半对数坐标纸上绘出标准曲线，根据样品的 OD 值由标准曲线查出相应的浓度；再乘以稀释倍数；或用标准物的浓度与 OD 值计算出标准曲线的直线回归方程式，将样品的 OD 值代入方程式，计算出样品浓度，再乘以稀释倍数，即为样品的实际浓度。

注意事项

1. **本操作说明也适用于 48T 试剂盒， 48T 试剂盒中酶联板、标准品、生物素标记抗体及辣根过氧化物酶标记亲和素减半。**
2. 当混合蛋白溶液时应尽量轻缓，避免起泡。
3. 洗涤过程非常重要，不充分的洗涤易造成假阳性。
4. 一次加样时间最好控制在 5 分钟内，如标本数量多，推荐使用排枪加样。
5. 请每次测定的同时做标准曲线，最好做复孔。
6. 如标本中待测物质含量过高，请先稀释后再测定，计算时请最后乘以稀释倍数。
7. 在配制标准品、检测溶液工作液时，请以相应的稀释液配制，不能混淆。
8. 底物请避光保存。
9. 不要用其它生产厂家的试剂替换试剂盒中的试剂。