MULTI SCIENCES
联科生物

Human IL-12p70 High Sensitivity ELISA Kit

This package insert must be read entirely before using this product. For proper performance, use the insert provided with each individual kit received.

Catalog Number

EK112HS - 48

EK112HS - 96

EK112HS - 192

EK112HS - 480

EK112HS - 960

For the quantitative determination of human Interleukin 12p70 (IL-12p70) concentration in cell culture supernates, serum and plasma.

For research use only.

Not for use in diagnostic procedures.

07/2017

Table of Contents

Introduction

Description	1
Principle of the Assay	1
Limitations of the Procedure	1

General Information

Materials Provided	3
Other Supplies required	3
Storage	5
Precaution	7
Technical Hints	9

Assay Protocol

Sample Collection and Storage	9
Reagent Preparation	11,13
Assay Procedure	15

Analysis

Calculation of Results	17
Typical Data	17
Sensitivity	19
Precision	19
Recovery	21
Linearity	21
Calibration	21
Sample Values	23
Specificity	23
Assay Procedure Summary	25

目录

产品介绍

背景介绍	2
检测原理	2
试剂盒检测的局限	2

基本信息

试剂盒提供的材料	4
未提供的材料设备	4
贮存	6
注意事项	8
技术要点	10

检测步骤

样本的收集和贮存	10
试剂准备	12, 14
检测步骤	16

分析

结果计算	18
典型数据	18
灵敏度	20
精密度	20
回收率	22
稀释线性	22
校准	22
样本值	24
特异性	24
检测步骤概要	26

DESCRIPTION

Interleukin 12 (IL-12) is an interleukin that is naturally produced by dendritic cells, macrophages, neutrophils human B-lymphoblastoid cells (NC-37) in response to antigenic stimulation. IL-12 is composed of a bundle of four alpha helices. It is a heterodimeric cytokine encoded by two separate genes, IL-12A (p35) and IL-12B (p40). The active heterodimer (referred to as 'p70'), and a homodimer of p40 are formed following protein synthesis.

IL-12 is involved in the differentiation of naive T cells into Th1 cells. It is known as a T cell-stimulating factor, which can stimulate the growth and function of T cells. IL-12 has anti-angiogenic activity, and plays an important role in the activities of natural killer cells and T lymphocytes. IL-12 is linked with autoimmunity as well.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-12p70 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and IL-12p70 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-linked detect antibody specific for IL-12p70 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, streptavidin-HRP is added. After washing, amplification reagent is added to the wells. Following incubation any unbound substances is removed during a wash step and streptavidin-HRP is added. After washing, substrate solution is added to the wells and color develops in proportion to the amount of IL-12p70 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE KIT

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

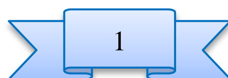
The kit should not be used beyond the expiration date on the kit label.

Do not mix or substitute reagents with those from other lots or sources.

If samples generate values higher than the highest standard, dilute the samples with Assay Buffer and repeat the assay. If cell culture supernate samples require large dilution, perform an intermediate dilution in culture medium.

Any variation in standard dilution, operator, pipetting technique, washing techniques, incubation time or temperature, and kit age can cause variation in binding.

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.



背景介绍

白细胞介素 12 (IL-12)由树突状细胞、巨噬细胞、中性粒细胞和人 B 淋巴母细胞株 (NC-37)在响应抗原刺激时自然产生。IL-12 由 4 个 a 螺旋组成，是一个由 2 种独立基因--- IL-12A (p35)和 IL-12B (p40)编码形成的异源二聚体细胞因子。蛋白合成之后可形成有生物活性的异源二聚体(p70)和同源二聚体 p40。

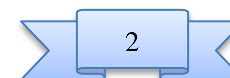
IL-12 参与初始 T 细胞向 Th1 细胞分化的过程，作为 T 细胞刺激因子，它可刺激 T 细胞的生长及其功能发挥。IL-12 具有抗血管生成活性，对自然杀伤细胞和 T 淋巴细胞活性有重要作用。同样地，IL-12 与自身免疫相关。

检测原理

本试剂盒采用双抗体夹心酶联免疫吸附检测技术。特异性抗人 IL-12p70 单克隆抗体预包被在高亲和力的酶标板上。酶标板孔中加入标准品和待测样本，经过孵育，样本中存在的 IL-12p70 与固相抗体结合。洗涤去除未结合的物质后，加入生物素化的检测抗体孵育。洗涤去除未结合的生物素化的抗体，加入辣根过氧化物酶标记的链霉亲和素 (Streptavidin-HRP)。洗涤后，加入信号增强剂孵育，洗涤去除未结合的物质后，再次加入 streptavidin-HRP。洗涤后，加入显色底物 TMB，避光显色。颜色反应的深浅与样本中 IL-12p70 的浓度成正比。加入终止液终止反应，在 450 nm 波长(参考波长 570 - 630 nm)测定吸光度值。

试剂盒检测的局限

1. 本试剂盒用于科学研究，非诊断试剂，不能用于临床诊断。
2. 请在本试剂盒标记的有效期内使用。
3. 试剂盒的试剂不能与其他批号的试剂或其他来源的试剂混合使用。
4. 如果样本值高于标准曲线最高浓度，请用检测缓冲液稀释样本，并重新检测。如果细胞培养上清样本需要较大的稀释倍数，请用细胞培养基进行适度稀释。
5. 任何标准品稀释、操作人员、移液技术、洗涤技术、孵育温度、试剂盒保存时间的改变，都将影响结合反应。
6. 本试剂盒在设计上去除或降低了生物学样本中的一些内源性干扰因素，并非所有可能的影响因素都已经去除。



MATERIALS PROVIDED

1. **IL-12p70 Microplate:** 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human IL-12p70.
2. **IL-12p70 Standard:** Recombinant human IL-12p70 in a buffered protein base with preservatives; lyophilized.
3. **IL-12p70 Detect Antibody:** Biotin-conjugate anti-human IL-12p70 detect antibody; 100× liquid.
4. **Standard Diluent:** In some, very rare cases, an insoluble precipitate of stabilizing protein has been seen in the Standard Diluent vial. This precipitate does not interfere in any way with the performance of the test and can thus be ignored.
5. **Streptavidin-HRP:** 100× liquid.
6. **Amplification Reagent Concentrate:** 100× liquid.
7. **Amplification Diluent.**
8. **Assay Buffer (10×):** PBS with 0.5 % Tween-20 and 5 % BSA.
9. **Substrate:** TMB (tetramethyl-benzidine).
10. **Stop Solution:** 0.18M sulfuric acid.
11. **Wash Buffer (20×):** PBS with 1 % Tween-20.
12. **Plate Covers**.

All reagents should be stored at 2 - 8℃.

OTHER SUPPLIES REQUIRED

1. **Microplate reader** capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.
2. **Pipettes and pipette tips**.
3. 50 µl to 300 µl adjustable **multichannel micropipette** with disposable tips.
4. Multichannel micropipette **reservoir.**
5. **Beakers, flasks, cylinders** necessary for preparation of reagents.
6. **Deionized or distilled water.**
7. **Polypropylene** test tubes for dilution.

试剂盒提供的材料

组分	编号	EK112HS-48	EK112HS-96	EK112HS-192	EK112HS-480	EK112HS-960
预包被酶标板	EK112P	48T	96T	192T	480T	960T
标准品	EK112HSS	1 vial	2 vial	4 vial	10 vial	20 vial
检测抗体	EK112HSD	1 vial	1 vial	2 vial	5 vial	10 vial
标准品稀释液	EG020	5 ml	5 ml	10 ml	25 ml	50 ml
辣根过氧化物酶标记的链霉亲和素	E0290	1 vial	1 vial	2 vial	5 vial	10 vial
信号增强剂浓缩液	BT0300010	1 vial	1 vial	2 vial	5 vial	10 vial
信号增强剂稀释液	TBTD	12 ml	12 ml	24 ml	60 ml	120 ml
10×检测缓冲液	E0310	5 ml	5 ml	10 ml	25 ml	50 ml
显色底物 TMB	E0230	11 ml	11 ml	22 ml	55 ml	110 ml
终止液	E0300	11 ml	11 ml	22 ml	55 ml	110 ml
20×洗液	E0281	50 ml	50 ml	100 ml	250 ml	500 ml
封板膜	E0200	10	10	20	50	100

未提供的材料设备

1. 能够检测 450 nm 吸光度的酶标仪，参考波长 570 nm 或 630 nm。
2. 移液器及枪头
3. 50 - 300 µl 可调多道移液器及一次性枪头
4. 多道移液器加样槽
5. 准备试剂用的试管、离心管、量筒等
6. 蒸馏水或去离子水
7. 稀释用聚丙烯试管

STORAGE

Store kit reagents between 2 and 8℃. Immediately after use remaining reagents should be returned to cold storage (2 to 8℃). Expiry of the kit and reagents is stated on labels.
Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Unopened kit		Store at 2 - 8℃.
Opened/ Reconstituted Reagents	Diluted Wash Buffer Diluted Assay Buffer Stop Solution Standard Diluent Substrate TMB Detect Antibody Streptavidin-HRP Amplification Reagent Concentrate Amplification Diluent	May be stored for up to 1 month at 2 - 8℃.
	Standard	For up to 1 month at ≤ -20℃ in a manual defrost freezer. Discard after use.
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8℃.

Provided this is within the expiration date of the kit.

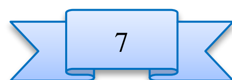
贮存

试剂盒保存于 2 - 8℃，有效期标注于标签上。只有恰当保存的试剂才是有保证的。如果试剂盒的组分需要再次使用，请确定上一次使用之后试剂没有污染。

未开封试剂盒		贮存于 2 - 8℃。请在有效期内使用。
打开的试剂盒或重组试剂	1×洗液 1×检测缓冲液 终止液 标准品稀释液 底物 TMB 检测抗体 辣根过氧化物酶标记的链霉亲和素 信号增强剂浓缩液 信号增强剂稀释液	在 2 - 8℃，大约可以贮存 1 个月。
	标准品	置于-20℃，大约可贮存 1 个月。 使用后丢弃。
	预包被酶标板	未使用的板条请放回铝箔袋，封好封口。 在 2 - 8℃，大约可贮存 1 个月。

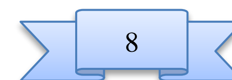
PRECAUTION

1. All chemicals should be considered as potentially hazardous.
2. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
3. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
4. The Stop Solution provided with this kit is an acid solution. Wear eyes, hand, face, and clothing protection when using this material.
5. Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
6. Do not mix or substitute reagents with those from other lots or other sources.
7. Do not use kit reagents beyond expiration date on label.
8. Do not expose kit reagents to strong light during storage or incubation.
9. Do not eat or smoke in areas where kit reagents or samples are handled.
10. Avoid contact of skin or mucous membranes with kit reagents or specimens.
11. Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
12. Avoid contact of substrate solution with oxidizing agents and metal.
13. Avoid splashing or generation of aerosols.
14. In order to avoid microbial contamination or cross- contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
15. Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
16. Exposure to acid inactivates the conjugate.
17. Glass-distilled water or deionized water must be used for reagent preparation.
18. Substrate solution must be at room temperature prior to use.
19. Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5℃.
20. Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0 % sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.
21. In some cases, an insoluble precipitate of stabilizing protein has been seen in the Standard Diluent. This precipitate does not interfere in any way with the performance of the test and can thus be ignored. Or remove precipitate by centrifuging at 6000 g for 5 minutes.



注意事项

1. 所有的化学试剂理应被认为具有潜在危害。
2. 联科生物推荐只有经过良好实验室培训的工作人员方可操作本试剂盒。操作时请佩戴合适的防护设施，例如白大衣、乳胶手套、安全眼镜等。
3. 请避免试剂接触皮肤和眼睛。如不慎接触，请立即用大量清水清洗。
4. 试剂盒中的终止液为酸性溶液，在使用终止液时，请佩戴防护服，及防护眼睛、手及面部的设施。
5. 本试剂盒用于科学研究，不能用于诊断治疗。
6. 请不要使用其他批号或其他来源的试剂替代本试剂盒中的试剂。
7. 请不要使用过期的试剂。
8. 在试剂盒的贮存或孵育过程请避免强光照射。
9. 在操作试剂盒或处理样本的区域请不要饮食。
10. 不要让试剂或样本接触皮肤和粘膜。
11. 在操作试剂盒或处理样本时请佩戴乳胶或一次性手套。
12. 显色底物避免与氧化试剂和金属接触。
13. 避免气溶胶的产生。
14. 为了避免微生物的污染，以及试剂与样本间的交叉污染，请使用一次性枪头。
15. 使用干净的容器配制试剂。
16. 暴露于酸性环境会抑制结合。
17. 试剂的准备必须使用蒸馏水或去离子水。
18. 显色底物在使用之前必须平衡至室温。
19. 样本可能含有传染性病原体，处理样本和可能的污染材料的首选方法是 121.5℃，最少 1 小时。
20. 液体废弃物的处理。不含酸的液体废弃物，加入 1.0 %的次氯酸钠，浸泡 30 分钟。含酸的液体废弃物，请先中和，再加入次氯酸钠。
21. 有时标准品稀释液中可观察到蛋白沉淀，该沉淀不影响使用，可以忽略。或者可通过 6000 g 离心 5 分钟去除沉淀。



TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. 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.
3. 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.
4. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
5. Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
6. Stop Solution should be added to the plate in the same order as the Substrate Solution.
7. 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.
8. It is recommended that all samples and standards be assayed in duplicate.
9. Take care not to scratch the inner surface of the microwells.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates – Remove particulates by centrifugation at 300 g for 10 minutes and assay immediately or aliquot and store samples at ≤ -20℃.

Serum – Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000 g. Remove serum and assay immediately or aliquot and store samples at ≤ -20℃.

Plasma – Collect plasma using EDTA, citrate or heparin as anticoagulant. Centrifuge at 1000 g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20℃.

Other biological samples might be suitable for use in the assay. Cell culture supernates, serum and plasma were tested with this assay.

Note: Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20℃ to avoid loss of bioactive human IL-12p70. If samples are to be run within 24 hours, they may be stored at 2 to 8℃.

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

技术要点

1. 重溶或者混合蛋白的时候，始终避免气泡产生。
2. 避免交叉污染，在进行标准品加样、样本加样，以及不同试剂加样的时候，请更换枪头。不同的试剂，使用不同的加样槽。
3. 在应用自动洗板机的时候，加入洗液之后，请设置一个 30 秒的浸泡程序，或者在不同的洗涤步骤对微孔板做 180 度的掉转，这样可以提高分析的准确度。
4. 为保证结果的精确性，孵育时封好封板膜。
5. 显色底物在添加之前应该是无色的。保持显色底物始终处于避光状态。
6. 终止液的添加顺序应该与显色底物的添加顺序相同。
7. 添加终止液之后，底物的颜色应该由蓝色转变为黄色。如果底物呈现绿色，说明终止液与显色底物没有充分混匀。
8. 推荐所有的检测样本和标准品在检测中设复孔。
9. 在任何情况下，避免接触微孔板的内表面。

样本采集与贮存

细胞培养上清

300 g 离心 10 分钟去除沉淀物，即刻检测，或者分装，-20℃以下贮存。

血清样本

分离管分离血清。在 1000 g 离心之前，使血样凝集 30 分钟。吸取血清样本之后即刻检测，或者分装，-20℃以下贮存。

血浆样本

EDTA、枸橼酸钠或肝素抗凝收集血浆样本。1000 g 离心 3 0 分钟收集样本。即刻检测，或者分装，-20℃以下贮存。

本试剂盒可能适用于其它生物学样本。细胞培养上清、血清和血浆已经过验证。

注意：检测前，样本中可见的沉淀必须去除。不要使用严重溶血或高血脂的样本。

样本应分装并贮存于-20℃，以避免人 IL-12p70 活性的丢失。如果在 24 小时内检测，样本可以存放在 2 - 8℃。

避免样本的反复冻融。在检测前，冷冻样本应该缓慢地恢复至室温，轻柔地混匀。

REAGENT PREPARATION

Bring all reagents and samples to room temperature before use.

If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

Wash Buffer (1×)

Pour entire contents (50 ml) of the **Wash Buffer (20×)** into a clean 1000 ml graduated cylinder. Bring to final volume of 1000 ml with glass-distilled or deionized water.

Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2 to 25℃. Please note that Wash Buffer (1×) is stable for 30 days.

Assay Buffer (1×)

Pour the entire contents (5 ml) of the **Assay Buffer (10×)** into a clean 100 ml graduated cylinder. Bring to final volume of 50 ml with distilled water. Mix gently to avoid foaming.

Store at 2 to 8℃. Please note that the Assay Buffer (1×) is stable for 30 days.

Detect Antibody

Mix well prior to making dilutions.

Make a **1：100** dilution of the concentrated **Detect Antibody** solution with Assay Buffer (1×) in a clean plastic tube as needed according to the Standards and samples.

Note: Detect Antibody should be used within 30 minutes after dilution.

Streptavidin-HRP

Mix well prior to making dilutions.

Make a **1：100** dilution of the concentrated **Streptavidin-HRP** solution with Assay Buffer (1×) in a clean plastic tube as needed according to the Standards and samples.

Note: Streptavidin-HRP should be used within 30 minutes after dilution.

Amplification Reagent Concentrate

Mix well prior to making dilutions.

Make a **1：100** dilution of the concentrated **Amplification Reagent** solution with **Amplification Diluent** in a clean plastic tube as needed according to the Standards and samples.

Note: Amplification Reagent should be used within 30 minutes after dilution.

Sample Dilution

If your samples need to be diluted, Assay Buffer (1×) is used for dilution of serum/plasma samples, and cell culture medium is used for dilution of culture supernates.

试剂准备

检测前请将所有的试剂、样本恢复至室温。

如果浓缩的试剂出现结晶，37℃温浴，直至结晶全部溶解。

1×洗液

吸取 20×浓缩洗液 50 ml 至 1 L 的量筒，加蒸馏水或去离子水至 1000 ml，轻轻混匀，避免泡沫。

转移至干净瓶内。2 - 25℃贮存，1×洗液可稳定 30 天。

1×检测缓冲液

吸取 10×浓缩检测缓冲液 5 ml 至 100 ml 量筒，加蒸馏水或去离子水至 50 ml，轻轻混匀，避免泡沫。

2 - 8℃贮存，1×检测缓冲液可稳定保存 30 天。

检测抗体

稀释前充分混匀。根据标准品和待测样本的数量，用 1×检测缓冲液按 **1：100** 稀释浓缩的检测抗体。

注意：请在 30 分钟内使用稀释后的检测抗体。

辣根过氧化物酶标记的链霉亲和素

稀释前充分混匀。

根据标准品和待测样本的数量，用 1×检测缓冲液按 **1：100** 稀释浓缩的辣根过氧化物酶标记的链霉亲和素。

注意：请在 30 分钟内使用稀释后的辣根过氧化物酶标记的链霉亲和素。

信号增强剂浓缩液

稀释前充分混匀。

根据标准品和待测样本的数量，用信号增强剂稀释液按 **1：100** 稀释浓缩的信号增强剂。

注意：请在 30 分钟内使用稀释后的信号增强剂。

样本稀释

如果样本需要稀释，请用试剂盒提供的 1×检测缓冲液稀释血清/血浆样本，用细胞培养基稀释细胞培养上清。

Human IL-12p70 Standard

Reconstitute **Human IL-12p70 Standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 50 pg/ml).

Allow the standard to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions.

Use polypropylene tubes.

For serum/plasma samples, the concentrated human IL-12p70 standard 250 µl + 250 µl Standard Diluent serves as the high standard (25 pg/ml). Pipette 250 µl of Standard Diluent into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Standard Diluent serves as the zero standard (0 pg/ml).

For cell culture supernates, the concentrated human IL-12p70 standard 250 µl + 250 µl cell culture medium serves as the high standard (25 pg/ml). Pipette 250 µl of cell culture medium into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Cell culture medium serves as the zero standard (0 pg/ml).

人 IL-12p70 标准品

用蒸馏水或去离子水重溶人 IL-12p70 标准品，重溶体积标注在人 IL-12p70 标准品的标签上。轻柔地涡旋震荡，确保充分混匀，重溶后标准品的浓度为 50 pg/ml。重溶后静置 10 - 30 分钟。稀释前充分混匀。

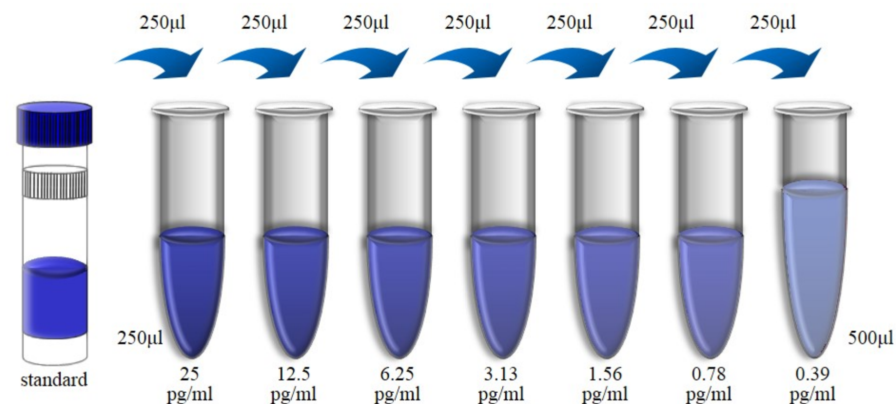
请使用聚丙烯管进行标准品稀释。

血清/血浆样本标准曲线的制作：

取 250 µl 浓缩的人 IL-12p70 标准品，加入 250 µl 标准品稀释液，作为标准曲线的最高浓度 (25 pg/ml)。在每一个试管中加入 250 µl 标准品稀释液。使用高浓度标准品做 1：1 系列稀释。每次移液时，请确保充分混匀。以标准品稀释液作为标准曲线的零浓度。

细胞培养上清样本标准曲线的制作：

取 250 µl 浓缩的人 IL-12p70 标准品，加入 250 µl 细胞培养基，作为标准曲线的最高浓度 (25 pg/ml)。在每一个试管中加入 250 µl 细胞培养基。使用高浓度标准品做 1：1 系列稀释。每次移液时，确保充分混匀。以细胞培养基作为标准曲线的零浓度。



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 300 µl Wash Buffer (1×) per well, and allow the Wash Buffer to sit in the wells for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance! Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. **Do not allow wells to dry.**
4. Add 100 µl of 2-fold diluted Standard to Standard well. Add 100 µl of Standard Diluent to Blank well.
5. Add 80 µl of Assay Buffer (1×) and 20 µl sample to the sample well. Ensure reagent addition in step 4 and 5 is uninterrupted and completed within 15 minutes.
6. Cover with an adhesive strip. Incubate at room temperature (18 to 25℃) for 1.5 hours on a microplate shaker set at 300 rpm.
7. Aspirate each well and wash, repeating the process five times for a total six washes. Wash by filling each well with 300µl Wash Buffer (1×). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
8. Add 100 µl of diluted Detect Antibody to each well. Cover with a new adhesive strip. Incubate at room temperature (18 to 25℃) for 30 minutes on a microplate shaker set at 300 rpm.
9. Repeat aspiration/wash as in step 7.
10. Add 100 µl of diluted Streptavidin-HRP to each well. Cover with a new adhesive strip. Incubate at room temperature (18 to 25℃) for 30 minutes on a microplate shaker set at 300 rpm.
11. Repeat aspiration/wash as in step 7.
12. Add 100 µl of diluted Amplification Reagent to each well. Cover with a new adhesive strip. Incubate at room temperature (18 to 25℃) for exactly 15 minutes on a microplate shaker set at 300 rpm.
13. Repeat aspiration/wash as in step 7.
14. Add 100 µl of diluted Streptavidin-HRP to each well. Cover with a new adhesive strip. Incubate at room temperature (18 to 25℃) for exactly 15 minutes on a microplate shaker set at 300 rpm.
15. Repeat aspiration/wash as in step 7.
16. Add 100 µl of Substrate Solution to each well. Incubate for 5 - 30 minutes at room temperature. Protect from light.
17. Add 100 µl of Stop Solution to each well. The color in the well should change from blue to yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
18. Determine the optical density within 30 minutes, using microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

检测步骤

检测之前请将所有的试剂、样本平衡至室温。

1. 准备好所有需要的试剂及工作浓度标准品。
2. 将不需要的板条拆卸下来，放回装有干燥剂的铝箔袋，重新封好封口。
3. 加入 300 µl 1×洗液静置浸泡 30 秒。为了获得理想的实验结果浸泡是必须的。弃掉洗液之后，在吸水纸上将微孔板拍干。洗板完成之后，请立即使用微孔板，不要让微孔板干燥。
4. 标准品孔加入 100 µl 2 倍倍比稀释的标准品。空白孔加入 100 µl 标准品稀释液。
5. 样本孔加入 80 µl 1×检测缓冲液和 20 µl 样本。保证步骤 4、5 连续加样，不要间断。加样过程在 15 分钟内完成。
6. 使用封板膜封板。300 转/分钟振荡，室温孵育 1.5 小时。
7. 弃掉液体，每孔加入 300 µl 洗液洗板，洗涤 6 次。每次洗板，在吸水纸上拍干。为获得理想的实验性能，必须彻底移除残留液体。
8. 每孔加入 100 µl 稀释的检测抗体。使用封板膜封板。300 转/分钟振荡，室温孵育 30 分钟。
9. 重复步骤 7。
10. 每孔加入 100 µl 稀释的辣根过氧化物酶标记的链霉亲和素。使用新的封板膜封板。300 转/分钟振荡，室温孵育 30 分钟。
11. 重复步骤 7。
12. 每孔加入 100 µl 稀释的信号增强剂。使用新的封板膜封板。300 转/分钟振荡，室温精确孵育 15 分钟。
13. 重复步骤 7。
14. 每孔加入 100 µl 稀释的辣根过氧化物酶标记的链霉亲和素。使用新的封板膜封板。300 转/分钟振荡，室温精确孵育 15 分钟。
15. 重复步骤 7。
16. 每孔加入 100 µl 显色底物 TMB，避光，室温孵育 5 - 30 分钟。
17. 每孔加入 100 µl 终止液。颜色由蓝色变为黄色。如果颜色呈现绿色或者颜色的变化明显不均匀，请轻轻叩击板框，充分混匀。
18. 在 30 分钟之内，使用酶标仪进行双波长检测，测定 450 nm 最大吸收波长和 570 nm 或 630 nm 参考波长下的 OD 值。校准后的 OD 值为 450 nm 的测定值减去 570 nm 或 630 nm 的测定值。仅使用 450 nm 测定会导致 OD 值偏高，并且准确度降低。

CALCULATION OF RESULTS

Average the duplicate readings for each standards and sample and subtract the average zero standard optical density.

Standard Concentration as horizontal axis, OD Value as the vertical axis, regressing the data, create a standard curve using computer software. The data may be linearized by plotting the log of the IL-12p70 concentrations versus the log of the OD and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Note: The finally concentration of top standard is 25 pg/ml. If instruction in this protocol have been followed samples have been diluted 1:4 (20 µl sample + 80 µl Assay Buffer), the concentration read from the standard curve must be multiplied by the dilution factor (×5).

If samples have been diluted following the instruction, the final dilution factor is 5. If sample have been diluted by other means, the concentration read from the standard curve must be multiplied by the appropriate dilution factor.

TYPICAL DATA

A standard curve should be generated for each set samples assayed. This standard curve is provided for demonstration only.

pg/ml	O.D.		Average	Corrected
0.00	0.061	0.059	0.060	
0.39	0.101	0.090	0.096	0.036
0.78	0.136	0.132	0.134	0.074
1.56	0.205	0.189	0.197	0.137
3.13	0.348	0.297	0.323	0.263
6.25	0.627	0.542	0.585	0.525
12.5	1.081	1.143	1.112	1.052
25.00	2.275	2.087	2.181	2.121

结果计算

计算标准品和样本的平均 OD 值，然后减去零浓度标准品的 OD 值。

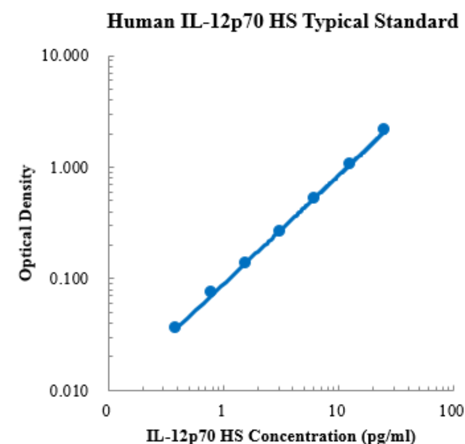
以标准品浓度为横坐标，OD 值为纵坐标，用计算机软件进行回归拟合生成标准曲线。回归分析确定最佳拟合曲线。通过对浓度值和 OD 值取对数拟合，可以对标准曲线进行线性化。此过程可能可以得到更多样本的浓度，但数据的准确度会降低一些。

注意：标准曲线最高浓度点的终浓度为 25 pg/ml。如果完全按照说明书的步骤操作（20 µl 样本 ＋80 µl 检测缓冲液），计算样本浓度时请乘以稀释因子 5。

如果样本按照说明书进行了稀释，最终的稀释倍数为 5。如果样本进行了其它方式的稀释，计算样本浓度时请乘以相应的稀释倍数。

典型数据

每次检测，每块酶标板都必须设立标准曲线。下面的标准曲线仅作为示例参考。



SENSITIVITY

The minimum detectable dose (MDD) of IL-12p70 is typically less than 0.04 pg/ml (mean of 6 independent assays).
The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in six separate assays to assess inter-assay precision

灵敏度

人 IL-12p70 的最低可检测浓度为 0.04 pg/ml (6 次独立实验的平均值)。
10 个零标准品浓度 OD 的平均值加上两倍 SD，计算最低可检测浓度。

精密度

酶标板内精密度
3 个已知浓度的样本酶标板内重复测定 20 次，评估酶标板内的精密度。

酶标板间精密度
3 个已知浓度的样本酶标板间重复检测 6 次，评估酶标板间的精密度。

	酶标板内精密度			酶标板间精密度		
样本	1	2	3	1	2	3
	20	20	20	6	6	6
平均值 (pg/ml)	0.43	1.66	5.77	0.64	1.91	5.83
标准差	0.02	0.08	0.28	0.04	0.08	0.27
变异系数 (%)	4.7	4.8	4.9	6.3	4.2	4.6

RECOVERY

The spike recovery was evaluated by spiking 3 levels of human IL-12p70 into five health human serum samples. The un-spiked serum was used as blank in these experiments.
The recovery ranged from 91 % to 104 % with an overall mean recovery of 97 %.

LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of IL-12p70 in human serum and diluted with Standard Diluent to produce samples with values within the dynamic range of the assay.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human IL-12p70 produced at MultiSciences.

回收率

5 份健康人血清加入 3 个不同浓度水平的人 IL-12p70，未加人 IL-12p70 的血清作为本底，计算回收率。回收率的范围从 91 %至 104 %，平均回收率为 97 %。

稀释线性

5 份健康人血清加入高浓度的人 IL-12p70，并在标准曲线的动力学范围内进行系列稀释，评估检测的线性。

	平均值 (%)	范围 (%)
1:2	87	80 - 93
1:4	102	94 - 110
1:8	108	98 - 117
1:16	98	87 - 108

校准

本试剂盒的标准品为联科生物校准的高纯度重组人 IL-12p70。

SAMPLE VALUES

Serum/Plasma – Thirty samples from apparently healthy volunteers were evaluated for the presence of IL-12p70 in this assay. No medical histories were available for the donors used in this study.

Sample Matrix	Number of Samples Evaluated	Range (pg/ml)	Detectable (%)	Mean of Detectable (pg/ml)
Serum	30	0.2 - 2.2	100	1.1

Note: The sample range is non-physiological range. The sample range of healthy human will difference according to geographical, ethic, sample preparation, and testing personnel, equipment varies. The above information is only reference.

SPECIFICITY

This assay recognizes both natural and recombinant human IL-12p70. The factors listed below were prepared at 1 ng/ml in Standard Diluent and assayed for cross-reactivity. Preparations of the following factors at 1 ng/ml in a mid-range rhIL-12p70 control were assayed for interference. No significant cross-reactivity or interference was observed.

Human		Mouse	Rat
IFN-γ	IL-18	IFN-γ	IFN-γ
IL-1β	IL-21	IL-1β	IL-1β
IL-2	IL-22	IL-2	IL-2
IL-4	IL-23	IL-4	IL-4
IL-5	MCP-1	IL-6	IL-6
IL-6	TGF-β1	IL-10	IL-10
IL-8	TNF-α	IL-17A	IL-17A
IL-10	VEGF	TNF-α	TNF-α
IL-17A			

样本值

应用本试剂盒，检测 30 份健康志愿者的血清样本，志愿者的用药史不详。

样本类型	检测样本数量	浓度范围 (pg/ml)	可测百分率 (%)	可测样本平均浓度 (pg/ml)
血清	30	0.2 - 2.2	100	1.1

注意：此样本值范围非生理值范围。健康人样本的浓度范围因地域、种族、样本制备以及检测人员、设备的不同而有所不同。以上数据仅供参考。

特异性

本试剂盒识别天然和重组人 IL-12p70。下述因子以 1 ng/ml 稀释于标准品稀释液中，评估交叉反应活性。下述因子以 1 ng/ml 稀释于中等浓度的人 IL-12p70 标准品中，评估干扰影响。没有观察到明显的交叉反应和干扰影响。

人		小鼠	大鼠
IFN-γ	IL-18	IFN-γ	IFN-γ
IL-1β	IL-21	IL-1β	IL-1β
IL-2	IL-22	IL-2	IL-2
IL-4	IL-23	IL-4	IL-4
IL-5	MCP-1	IL-6	IL-6
IL-6	TGF-β1	IL-10	IL-10
IL-8	TNF-α	IL-17A	IL-17A
IL-10	VEGF	TNF-α	TNF-α
IL-17A			

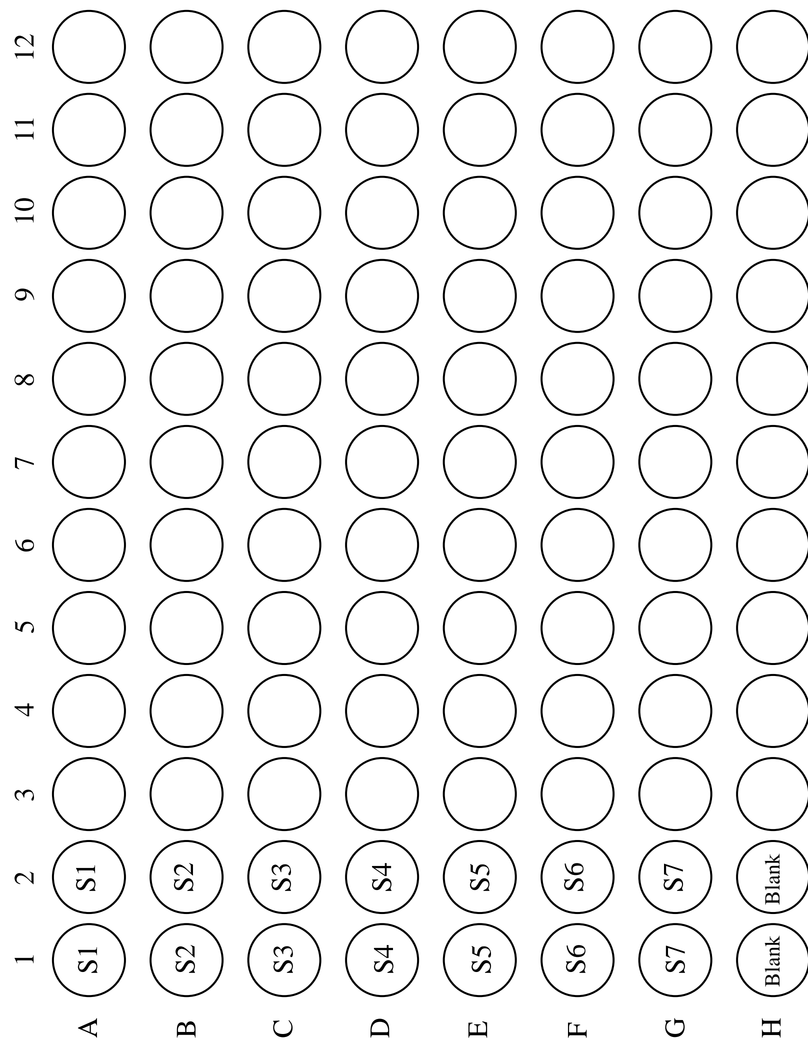
ASSAY PROCEDURE SUMMARY

1. Prepare all reagents and standards as directed.
2. Add 100 µl 2-fold diluted Standard to Standard well. Add 100 µl Standard Diluent to Blank well.
3. Add 80 µl Assay Buffer (1×) and 20 µl sample to the sample well. Step 2 and 3 should be completed within 15 minutes. Incubate for 1.5 hours at RT. Aspirate and wash 6 times.
4. Add 100 µl diluted Detect Antibody to each well. Incubate for 30 minutes at RT. Aspirate and wash 6 times.
5. Add 100 µl diluted Streptavidin-HRP to each well. Incubate for 30 minutes at RT. Aspirate and wash 6 times.
6. Add 100 µl diluted Amplification Reagent to each well. Incubate for exactly 15 minutes at RT.
7. Aspirate and wash 6 times.
8. Add 100 µl diluted Streptavidin-HRP to each well. Incubate for exactly 15 minutes at RT.
9. Aspirate and wash 6 times.
10. Add 100 µl Substrate Solution to each well. Incubate for 5 - 30 minutes at RT. Protect from light.
11. Add 100 µl Stop Solution to each well.
12. Read at 450 nm within 30 minutes. Correction 570 or 630 nm.

检测步骤概要

1．准备所有的试剂和标准品。
2．标准品孔加入 100 µl 2 倍倍比稀释的标准品。空白孔加入 100 µl 标准品稀释液。
3．样本孔加入 80 µl 1×检测缓冲液和 20 µl 样本。步骤 2、3 在 15 分钟内完成。室温孵育 1.5 小时。洗涤 6 次。
4．每孔加入 100 µl 稀释的检测抗体。室温孵育 30 分钟。洗涤 6 次。
5．每孔加入 100 µl 稀释的辣根过氧化物酶标记的链霉亲和素。室温孵育 30 分钟。洗涤 6 次。
6．每孔加入 100 µl 稀释的信号增强剂。室温精确孵育 15 分钟。
7．洗涤 6 次。
8．每孔加入 100 µl 稀释的辣根过氧化物酶标记的链霉亲和素。室温精确孵育 15 分钟。
9. 洗涤 6 次。
10. 每孔加入 100 µl 显色底物，避光，室温孵育 5 - 30 分钟。
11．每孔加入 100 µl 终止液。
12. 30 分钟内，在 450 nm 波长检测 OD 值，参考波长 570 nm 或 630 nm。

PLATE LAYOUT



NOTE