Human Histone H4 (HIST1H4A) ELISA Kit

Catalog Number. CSB-EL010429HU

For the quantitative determination of human histone H4 (HIST1H4A) concentrations in serum, plasma, tissue homogenates, cell lysates.

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

If You Have Problems

Technical Service Contact information

Phone: 86-27-87582341
Fax: 86-27-87196150
Email: tech@cusabio.com
Web: https://www.cusabio.com/

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for HIST1H4A has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any HIST1H4A present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for HIST1H4A is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of HIST1H4A bound in the initial step. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

37.5 pg/ml-2400 pg/ml.

SENSITIVITY

The minimum detectable dose of human HIST1H4A is typically less than 9.3 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. It was determined the mean O.D value of 20 replicates of the zero standard added by their three standard deviations.

SPECIFICITY

This assay has high sensitivity and excellent specificity for detection of human HIST1H4A. No significant cross-reactivity or interference between human HIST1H4A and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between human HIST1H4A and all the analogues, therefore, cross reaction may still exist.

PRECISION

Intra-assay Precision (Precision within an assay): CV%<8%
Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<10%
Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- **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 Sample Diluent and repeat the assay.
- Any variation in Sample 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.

MATERIALS PROVIDED

Reagents	Quantity
Assay plate (12 x 8 coated Microwells)	1(96 wells)
Standard (Freeze dried)	2
Biotin-antibody (100 x concentrate)	1 x 120 µl
HRP-avidin (100 x concentrate)	1 x 120 µl
Biotin-antibody Diluent	1 x 15 ml
HRP-avidin Diluent	1 x 15 ml
Sample Diluent	1 x 50 ml
Wash Buffer (25 x concentrate)	1 x 20 ml
TMB Substrate	1 x 10 ml
Stop Solution	1 x 10 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

STORAGE

<table>
<tr><td>Unopened kit</td><td colspan="2">Store at 2 - 8°C. Do not use the kit beyond the expiration date.</td></tr>
<tr><td rowspan="10">Opened kit</td><td>Coated assay plate</td><td>May be stored for up to 1 month at 2 - 8°C. Try to keep it in a sealed aluminum foil bag, and avoid the damp.</td></tr>
<tr><td>Standard</td><td rowspan="3">May be stored for up to 1 month at 2 - 8° C. If don’t make recent use, better keep it store at -20°C.</td></tr>
<tr><td>Biotin-antibody</td></tr>
<tr><td>HRP-avidin</td></tr>
<tr><td>Biotin-antibody Diluent</td><td rowspan="6">May be stored for up to 1 month at 2 - 8°C.</td></tr>
<tr><td>HRP-avidin Diluent</td></tr>
<tr><td>Sample Diluent</td></tr>
<tr><td>Wash Buffer</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

***Provided this is within the expiration date of the kit.**

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100ml and 500ml graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.

PRECAUTIONS

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

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.Centrifuge the sample again after thawing before the assay.
- **Plasma** Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g at 2 - 8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Centrifuge the sample again after thawing before the assay.
- **Tissue Homogenates** 100mg tissue was rinsed with 1X PBS, homogenized in 1 ml of 1X PBS and stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g, 2 - 8°C. The supernate was removed and assayed immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.
- **Cell Lysates** **(1) Adherent Cell**: Remove media and rinse cells once with ice-cold PBS (pH7.2-7.4). Scrape cells off the plate and transfer to an appropriate tube. Dilute cell suspension with 1x PBS (pH7.2-7.4), until cell concentration reached 100 million/ml. Then store overnight at -20°C. After two freeze-thaw cycles to break up the cell membranes, the cell lysates were centrifuged for 5 minutes at 5000 x g, 2 - 8°C. Collect the supernatant. Cell lysates should be assayed immediately or aliquotted and stored at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles. **(2) Suspension Cel**l: Collect cells with appropriate tube, centrifuge for 5 minutes at 1000 x g, 2 - 8°C. Remove the supernatant and resuspend cells with 1x PBS (pH7.2-7.4). Centrifuge for 5 minutes at 1000 x g, 2 - 8°C. Remove the supernatant. Dilute cell with 1x PBS (pH7.2-7.4), until cell concentration reached 100 million/ml. Then store overnight at -20°C. After two freeze-thaw cycles to

break up the cell membranes, the cell lysates were centrifuged for 5 minutes at 5000 x g, 2 - 8°C. Collect the supernatant. Cell lysates should be assayed immediately or aliquotted and stored at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Note:

1. CUSABIO is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1month) or -80°C (≤2month) to avoid loss of bioactivity and contamination.
3. Grossly hemolyzed samples are not suitable for use in this assay.
4. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
5. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
6. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
7. Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
8. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
9. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENT PREPARATION

Note:

- **Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.**
- Bring all reagents to room temperature (18-25°C) before use for 30min.
- Prepare fresh standard for each assay. Use within 4 hours and discard after use.
- Making serial dilution in the wells directly is not permitted.
- Please carefully reconstitute Standards according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10μl for once pipetting.
- Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

1. **Biotin-antibody (1x) -** Centrifuge the vial before opening.
   Biotin-antibody requires a 100-fold dilution. A suggested 100-fold dilution is 10 μl of **Biotin-antibody** + 990 μl of **Biotin-antibody Diluent**.

2. **HRP-avidin (1x) -** Centrifuge the vial before opening.
   HRP-avidin requires a 100-fold dilution. A suggested 100-fold dilution is 10 μl of **HRP-avidin** + 990 μl of **HRP-avidin Diluent**.

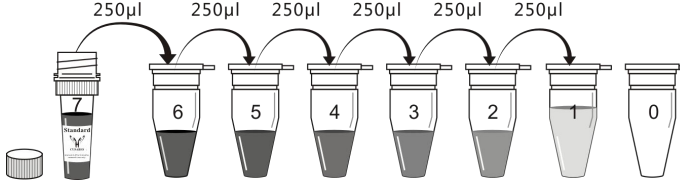
3. **Wash Buffer(1x)-** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).

4. **Standard**

Centrifuge the standard vial at 6000-10000rpm for 30s.

Reconstitute the **Standard** with 1.0 ml of **Sample Diluent**. Do not substitute other diluents. This reconstitution produces a stock solution of 2400 pg/ml. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 250 µl of **Sample Diluent** into each tube (S0-S6). Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (2400 pg/ml). **Sample Diluent** serves as the zero standard (0 pg/ml).



Tube	S7	S6	S5	S4	S3	S2	S1	S0
pg/ml	2400	1200	600	300	150	75	37.5	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Add 100µl of standard and sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at 37°C. A plate layout is provided to record standards and samples assayed.
4. Remove the liquid of each well, **don't wash.**
5. Add 100µl of **Biotin-antibody (1x)** to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37°C. (**Biotin-antibody (1x)** may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.)
6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, 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.
7. Add 100µl of **HRP-avidin (1x)** to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 1 hour at 37°C.
8. Repeat the aspiration/wash process for five times as in step 6.
9. Add 90µl of **TMB Substrate** to each well. Incubate for 15-30 minutes at 37°C. **Protect from light.**
10. Add 50µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.

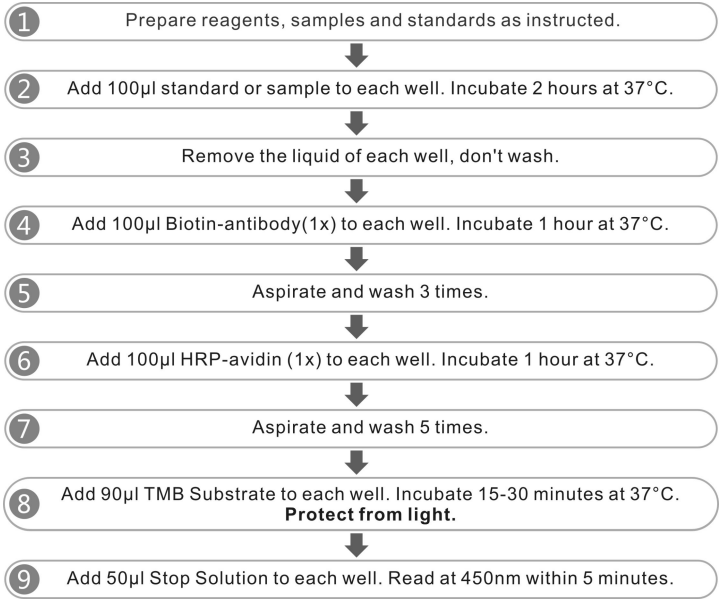
11. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. 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. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. 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.

5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. TMB Substrate is easily contaminated. TMB Substrate should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the TMB Substrate. 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 TMB Substrate.

ASSAY PROCEDURE SUMMARY



***Please determine whether the sample needs to be diluted or the optimal dilution factor based on preliminary experiment result.**

CALCULATION OF RESULTS

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

Average the duplicate readings for each standard 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 x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the HIST1H4A 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.

人组蛋白 H4 (HIST1H4A)酶联免疫试剂盒
使用说明书

【产品编号】CSB-EL010429HU

【预期应用】ELISA 法定量测定人血清、血浆、组织裂解液、细胞裂解液中 HIST1H4A 含量。

【产品性能指标】

1、 检测范围：37.5 pg/ml-2400 pg/ml

2、 灵敏度：9.3 pg/ml

3、 精密度：批内差 CV%<8%，批间差 CV%<10%

4、 特异性：本试剂盒特异性检测人 HIST1H4A，且与其他相关蛋白无交叉反应。

【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 HIST1H4A 抗体的微孔中依次加入标本或标准品、生物素化的抗 HIST1H4A 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。颜色的深浅和样本中的 HIST1H4A 呈正相关。用酶标仪在 450nm 波长下测定吸光度（OD 值），计算样本浓度。

【试剂盒组成成分】

组份	96T
酶标板 (Assay plate)	12 条×8 孔
标准品 (Standard)	2 瓶 (冻干品)
生物素标记抗体 (Biotin-antibody)	1 x 120 μl/瓶 (100×)
辣根过氧化物酶标记亲和素 (HRP-avidin)	1 x 120 μl/瓶 (100×)
生物素标记抗体稀释液 (Biotin-antibody Diluent)	1 x 15 ml/瓶
辣根过氧化物酶标记亲和素稀释液 (HRP-avidin Diluent)	1 x 15 ml/瓶
样本稀释液 (Sample Diluent)	1 x 50 ml/瓶
浓洗涤液 (Wash Buffer)	1 x 20 ml/瓶 (25×)
底物溶液 (TMB Substrate)	1 x 10 ml/瓶
终止液 (Stop Solution)	1 x 10 ml/瓶
板贴	4

【存储条件及有效期】

<table>
<tr><td>未开封试剂盒</td><td colspan="2">试剂盒避光保存于2-8℃。有效期为六个月。
请在试剂盒标注的有效日期内使用。</td></tr>
<tr><td rowspan="9">开封试剂盒</td><td>预包被的酶标板</td><td>酶标板打开后应置有干燥剂的铝箔袋中置于 2-8℃密封防潮保存。有效期内 2-8℃条件下最多可保存一个月。</td></tr>
<tr><td>标准品</td><td rowspan="3">有效期内2-8℃条件下最多可保存一个月。
若近期不使用，最好保存在-20°C。</td></tr>
<tr><td>生物素标记抗体</td></tr>
<tr><td>辣根过氧化物酶标记亲和素</td></tr>
<tr><td>生物素标记抗体稀释液</td><td rowspan="6">有效期内2-8℃条件下最多可保存一个月。</td></tr>
<tr><td>辣根过氧化物酶标记亲和素稀释液</td></tr>
<tr><td>样本稀释液</td></tr>
<tr><td>浓洗涤液</td></tr>
<tr><td>底物溶液</td></tr>
<tr><td>终止液</td></tr>
</table>

【所需试剂和器材】

标准规格酶标仪；高速离心机；电热恒温培养箱；干净的试管和离心管；容量瓶；

系列可调节移液器及吸头；多通道移液器；蒸馏水等

【样本采集及保存】

1、 血清：全血标本请于室温放置 2 小时或 4°C 过夜后于 2 - 8°C 1000 x 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°C 或-80°C 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 x 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°C 或-80°C 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。

3、 组织裂解液：取 100mg 组织，用 1X PBS 洗去血污。剪成小块放入组织研磨器（匀浆管）中，加入 1 ml 1X PBS，制成匀浆，然后置于-20°C 过夜。经过反复冻融 2 次处理破坏细胞膜后，将组织匀浆于 2 - 8°C 5000 x g 离心 5 分钟取上清。取适量上清液立即进行实验，

或将上清分装保存于-20°C 或-80°C。解冻后的样品应再次离心，然后检测。避免反复冻融。

4、 细胞裂解液：**(1)贴壁细胞**：将长满细胞的培养瓶放置在冰上，用吸液管吸出培养液。加入足够的冷的 PBS(pH7.2-7.4)在培养瓶中充分洗涤细胞表面，以洗去瓶中残留的培养基，倒掉 PBS。尽可能吸干残留的 PBS，尽量在冰上操作。将清洗过的细胞转移到合适的离心管中，用 1xPBS (pH7.2-7.4)稀释到浓度为 100 million/ml。然后置于-20°C 过夜。经过 2 轮反复冻融破坏细胞膜后，于 2 - 8°C 5000 x g 离心 5 分钟取上清，上清即可立即检测；或进行分装，并将标本放于-20°C 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)悬浮细胞**：使用合适的离心管收集细胞，于 2 - 8°C 1000 x g 离心 5 分钟去上清，使用 1xPBS (pH7.2-7.4)轻柔重悬细胞，再次采用 2 - 8°C 1000 x g 离心 5 分钟去上清，用 1xPBS (pH7.2-7.4)稀释到浓度为 100 million/ml。然后置于-20°C 过夜。经过 2 轮反复冻融破坏细胞膜后，于 2 - 8°C 5000 x g 离心 5 分钟取上清，上清即可立即检测；或进行分装，并将标本放于-20°C 保存，但应避免反复冻融。解冻后的样本应再次离心，去除沉淀，然后检测。

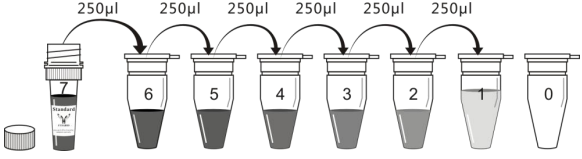
注：标本溶血会影响最后检测结果，因此溶血标本不宜进行此项检测。

【试剂配制】

1、 **标准品**

(1) 从试剂盒中取出一支标准品，于 6000-10000rpm 离心 30 秒。用 1ml 样本稀释液溶解，并用枪头对准冻存管底部反复吸打 5 次以助溶解，充分混匀得到标准品 S7，放置备用。

(2) 取 7 个 1.5 ml 离心管(S0-S6)依次排列，各加入 250μl 样本稀释液。吸取 250μl 标准品 S7 到第一个离心管中(S6)，轻轻吹打混匀。从 S6 中吸取 250μl 到第二个 EP 管中(S5)，轻轻吹打混匀。以此类推进行标准品的倍比稀释。S0 为样本稀释液。



【标准品浓度】

编号	S7	S6	S5	S4	S3	S2	S1	S0
pg/ml	2400	1200	600	300	150	75	37.5	0

2、 洗液工作液

浓洗涤液按1:25倍用去离子水进行稀释。例如用量筒量取240ml去离子水，倒入烧杯或其他洁净容器中，再量取10ml浓洗涤液，均匀加入，搅拌混匀，在临用前配妥。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、 生物素标记抗体工作液

生物素标记抗体液按1:100倍用生物素标记抗体稀释液进行稀释。如10μl生物素标记抗体加990μl生物素标记抗体稀释液，轻轻混匀，在临用前10分钟内配妥。

4、 辣根过氧化物酶标记亲和素工作液

辣根过氧化物酶标记亲和素按1:100倍用辣根过氧化物酶标记亲和素稀释液进行稀释。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，轻轻混匀，在临用前10分钟内配妥。

【重要提示】

1、 实验开始前，请提前配置好所有试剂。试剂或样本稀释时，均需混匀，混匀时尽量避免起泡。

2、 用户在初次使用试剂盒时，应将各种试剂管离心数分钟，以便管盖和管壁上的液体集中到管底。

3、 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。

4、 因实际装量多于标示装量，配制工作试剂请用精准的计量工具（移液枪或量筒等）量取，切勿不经量取直接使用。

5、 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稀释前根据预先计算好的每次实验所需的总量配制，实际配制时应多配制 0.1-0.4ml。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。

6、 标准品的稀释请勿于板中进行，标准品应于临用前 15 分钟内配制。为保证实验有效性，建议每次实验使用新的标准品。若保存得当，溶解后的标准品 S7 可在 2-8℃保存，7 天内使用有效。

【操作步骤】

1、 将各种试剂移至室温（18-25℃）平衡至少 30 分钟，按前述方法配制试剂，备用。

2、 加样：分别设标准品孔、待测样本孔。每孔分别加标准品或待测样本 100μl，轻轻晃动混匀，覆上板贴，37℃温育 2 小时。

3、 弃去液体，甩干，不用洗涤。

4、 每孔加生物素标记抗体工作液 100μl，覆上新的板贴，37℃温育 1 小时。

5、 弃去孔内液体，甩干，洗板 3 次。每次浸泡 2 分钟，200μl/孔，甩干。

6、 每孔加辣根过氧化物酶标记亲和素工作液 100μl，覆上新的板贴，37℃温育 1 小时。

7、 弃去孔内液体，甩干，洗板 5 次。每次浸泡 2 分钟，200μl/孔，甩干。

8、 依序每孔加底物溶液 90μl，37℃避光显色 15-30 分钟。

9、 依序每孔加终止溶液 50μl，终止反应。

10、在反应终止后 5 分钟内用酶标仪在 450nm 波长依序测量各孔的光密度（OD 值）。

【操作要点】

1、 为保证检测结果的准确性，建议标准品及样本均设双孔测定。每次检测均需做标准曲线。

2、 如标本中待测物质含量过高，请先用样本稀释液进行稀释，以使样本符合试剂盒的检测范围，最后计算时再乘以相应的稀释倍数。

3、 **加样**：加样时，请使用一次性的洁净吸头，避免交叉污染。加样时应尽量轻缓，避免起泡，将样本加于酶标板孔底部，切勿沿孔壁加样。一次加样时间最好控制在 10 分钟内，如标本数量多，推荐使用排枪加样。

4、 **温育**：为防止样本蒸发或污染，温育过程中酶标板必须覆上板贴，实验过程中酶标板应避免处于干燥的状态。温育过程中应随时观察温箱温度是否恒定于 37℃，及时调整。温育过程中，温箱不易开启太多次，以免影响温度平衡。

5、 **洗涤**：洗涤过程非常重要，不充分的洗涤易造成假阳性。

(1) 手工洗板方法：吸去（不可触及孔壁和孔底）或甩掉酶标板内的液体；在实验台上铺垫几层吸水纸，酶标板朝下轻拍几次；将推荐的洗涤缓冲液按 200μl/孔注入孔内，浸泡 2 分钟。根据操作步骤中所述，重复此过程数次。

(2) 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。

6、 **显色**：为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。可在加入底物溶液后每隔一段时间观察一下显色情况以控制反应时间（比如每隔 10 分钟）。当肉眼可见标准品前 3-4 孔有明显梯度蓝色，后 3-4 孔显色不明显时，即可加入终止液终止反应，此时蓝色立刻变为黄色。终止液的加入顺序应尽量与底物溶液的加入顺序相同。

7、 底物溶液应为浅蓝色或无色，如果颜色严重变深则必须弃用。底物溶液易受污染，请避光妥善保存。

【数据处理】

可将标准品及样本值减去 S0 孔数值后绘制曲线，如果设置复孔，则应取其平均值计算。以标准品的浓度为纵坐标（对数坐标），OD 值为横坐标（对数坐标），在对数坐标纸上绘出标准曲线。推荐使用专业制作曲线软件进行分析，可从我们的网站下载专业软件"Curve Expert"，并根据提示制作标准曲线。根据样本 OD 值，由标准曲线查出相应的浓度；或用标准品的浓度与 OD 值计算出标准曲线的回归方程式，将样本的 OD 值代入方程式，计算出样本浓度。若样本检测前进行过稀释，最后计算时需乘以相应的稀释倍数，即为样本的实际浓度。

【说明】

1、 本试剂盒仅供研究使用。

2、 中、英文说明书可能会有不一致之处，请以英文说明书为准。

3、 本操作说明也适用于 48T 试剂盒。48T 试剂盒中酶标板、标准品、生物素标记抗体及辣根过氧化物酶标记亲和素数量减半。

4、 不同批号试剂不能混用。不要用其它生产厂家的试剂替换试剂盒中的试剂。

5、 刚开启的酶标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。

Notes