SunLong Biotech Co.,LTD

Tel: 0086-571- 56623320 Fax:0086-571- 56623318

E-mail:sales@sunlongbiotech.com

www.sunlongbiotech.com

HEK 293 Host Cell Protein ELISA Kit

Cat No.:HP-HCP48T

48 Wells

Valid Period: 6 months

ELISA Kit for the quantitative Measurement of HEK 293 HCP Residues in Cell Culture Supernatants, Protein Purification Process, and End-Product

FOR RESEARCH, DEVELOPMENT AND MANUFACTURING USE ONLY!
NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!
PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

CONTENTS

1. APPLICATION

The kit is intended for the quantitative determination of residual host cell protein in Protein therapeutics by HEK 293 expression systems.

2. BACKGROUND

Human Embryonic Kidney 293 Cells, commonly known as HEK 293, is widely utilized as an expression of therapeutic proteins. The process of expressing target proteins, undergoing concomitant apoptosis, which release up to a thousand of host proteins into cell culture medium after the break of cells. These host proteins exhibit strong immunogenicity, which can lead to adverse toxicity or immune reactions, jeopardizing product safety and quality, and causing potential biological contamination. One of the aims of downstream processes in the production of biological medicinal products is to remove these potential hazards.

Therefore, it is essential to minimize the residual levels of host cell proteins (HCP), and in the downstream purification process development, a scientifically sound method for determining the concentration of HCP in finished or semi-finished products is necessary. Enzyme-linked immunosorbent assay (ELISA) has a high sensitivity and is therefore designated as the gold standard for HCP detection by regulatory agencies.

3. PRINCIPLE OF THE ASSAY

This ELISA kit is applied to the solid phase sandwich Enzyme-Linked Immunosorbent Assay. The microplate has been pre-coated with a capture antibody. The antigen to be measured and the HRP-conjugated detection antibody are then added to the microplate wells sequentially to form a capture antibody-antigen-detection antibody complex. After washing, the conjugates uninvolved in the reaction are removed, and then a TMB substrate solution is added to the wells to incubate. TMB substrate solution turns blue after the oxidation of HRP and finally turns yellow immediately after adding the stop solution. The color of the TMB substrate positively correlated with the antigen bound in the initial steps. Measure the absorbance (OD value) at 450nm using a microplate reader, and create a standard curve along with the corresponding concentrations. Then, by inputting the OD values of the samples into the standard curve equation, calculate the concentration of the target

protein in the sample.

4. KITS' ADVANCEMENT

1) High Coverage: The capture and detection antibody are both derived from rabbit, which have strong recognition of host-cell proteins (HCPs). Moreover, there is little individual variation within this species, ensuring high comparability and process stability.
2) High Antibody Titers: The antibodies used in the reagent kit are tested using the indirect method with Elisa, and the results indicate a titer of at least 10^7.
3) High Sensitivity: Serum antibody purification employs affinity purification to remove non-specific antibodies to the greatest extent.
4) High Stability: The production process uses a broad-spectrum protein stabilizer and microplate processing technology to enhance the stability and repeatability of the standard and microplate result.
5) Optimal Diluent Buffer: Using an optimized dilution solution can reduce non-specific adsorption during the sample detection process, resulting in very low background coloration that facilitates the observation of the concentration of the samples.

5. MATERIALS (Note: Storage at 2-8℃)

	Reagents	**Specification**	**Quantity**
1	Pre-Coated Microplate (Detachable)	48 wells	1 plate (Keep Sealed)
2	Standard (Stock Solution) -500ug/ml	30ul	1 tube
3	Detection antibody (100×)	100ul	1 tube
4	TMB Substrates	6ml	1 vial (Avoid Light)
5	Stop Solution	6ml	1 vial
6	Wash Solution (100×)	10ml	1 vial
7	Diluent Buffer (10×)	10ml	1 vial
8	Plate Sealer		4 pieces
9	Instruction Manual		1

6. EQUIPMENT REQUIRED BUT NOT PROVIDED

1) 10-1000 µl pipettor and disposable sterilized tips
2) Multichannel pipettor
3) 1L sterilized deionized water or ultrarpure water
4) Sterilized EP tubes
5) Absorbent Paper
6) Microplate reader
7) High-speed centrifuge
8) Mini Centrifuge
9) Microplate washer or washing bottles
10) Data analysis and graphing software

7. SPECIMEN COLLECTION AND STORAGE

Cell Culture Supernatant -Collect cultured cell, centrifuge them at 1000×g (or 3000 rpm) for 15 minutes, and collect the supernatant for immediate assay or store samples in aliquots at -20°C or -80°C.

NOTE:

- Samples should be aliquoted and must be stored at -20°C (less than 3 months) or -80°C (less than 6 months) to avoid loss of bioactivity and contamination. If samples are to be run within 24 hours, they may be stored at 2- 8°C. Avoid repeated freeze-thaw cycles.
- Some chemical solvents may interfere with this experiment, such as SDS, Triton, etc.
- The precipitates in the sample solution can interfere with ELISA. Make sure to centrifuge and remove them.
- Do not use heat-treated specimens.

8. REAGENT PREPARATION

1) Place all kits' components at room temperature for 30mins before using.
2) The preparation of Wash Solution (1×): Dilute 10 mL of Wash Solution (100×) with 990 mL of deionized water or ultrapure water to prepare 1000 mL of Wash Solution (1×). If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have

completely dissolved.

3) The preparation of Diluent Buffer: Dilute 10 mL of Diluent Buffer (10×) with 90 mL of deionized water or ultrapure water to prepare 100 mL of Diluent Buffer (1×) . If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. The buffer is used as the Diluent Buffer for standards, samples and detection antibodies.

9. ASSAY PROCEDURE

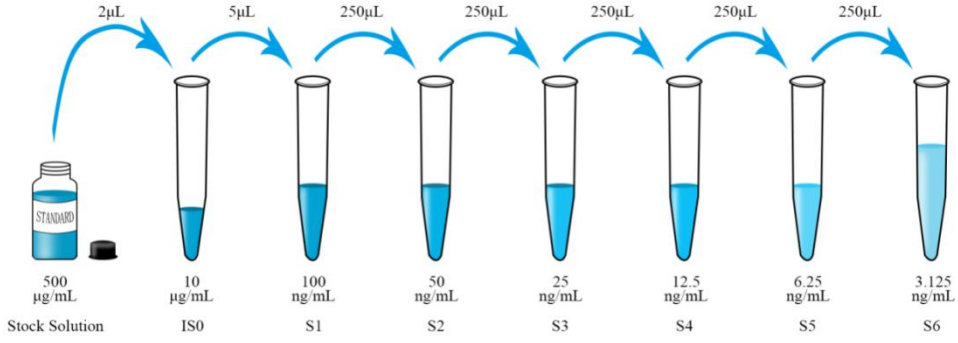
1) Remove the microplate from the foil bag, detach the desired number of strips from the plate, immediately reseal the remaining strips and store at 4°C. Then put the ready-for-use strips on a clean microplate frame, and start the experiment after the strips returned to room temperature (Note: The microplate frame can be reused). The standard may stick to the tube wall due to transport turbulence. Before use, gently shake it or centrifuge for about 2 seconds.

2) Prepare Standards: When preparing the standards, label 7 tubes IS0, S1-S6 and add a certain volume of diluent: IS0 (98ul), S1 (495ul), S2 to S6 (each 250ul).

Add 2ul of the Standard (Stock Solution-500ug/ml) into the tube labeled as IS0. Shake well and then take 5 ul IS0 (10ul/mL), and add it to tube S1. Shake well and then pipette 250ul Standard solution (100ng/ml) from S1 to S2. And then produce a 2-fold dilution series until S6 (see below). Secure the desired plate in the holder then add 100ul of diluted standards (S1-S6) or 100ul (diluted or undiluted) samples to appropriate wells.

For the Blank Control test, we recommend add 100ul Diluent Buffer to the well.

NOTE: There are 6 points of diluted standards, S1: 100ng/ml, S2: 50ng/ml, S3: 25ng/ml, S4: 12.5ng/ml, S5: 6.25ng/ml, S6: 3.125ng/ml.



3) Cover the plate with sealer, put it on the oscillator to mix and incubate for 90 mins at room temperature.

4) Automated Washing:

- Put 1000 ml Wash Solution (1×) into the washing bottle of the automated microplate washer for standby.
- Take out the microplate, and discard the incubation mixture. And then wash the microplate with the automated microplate washer for 5 times.
- After washing, invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no moisture appears.

Or Manual Washing:

- Put 1000 ml Wash Solution (1×) into the washing bottle for standby.
- Take out the microplate, and discard the incubation mixture. And then invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no moisture appears.
- Fill each well with 300ul Wash Solution (1×) by a muti-channel pipette, let stand for 20 seconds, then discard the contents, invert plate, and blot dry by hitting the plate onto absorbent paper. Repeat this washing step for 5 times. Note: There should be no moisture appears in the fifth washing step.

5) Prepare Detection Antibody: Extract 60ul of Detection antibody (100×), and add it into 6 ml Diluent Buffer to reach its working concentration (1×) and mix gently. Add 100ul of above diluted detection antibody to each well, cover the plate with a sealer, and put it on the oscillator to mix and incubate for 90 mins at room temperature.

6) Washing Step: Repeat the same procedure as step 4.

7) Add 100ul TMB substrate to each well. Cover the plate with a sealer, incubate at room temperature for about 15 mins. If the color is light, the reaction time can be extended appropriately, but not more than 30min.

8) Add 50ul of Stop Solution to each well to stop the reaction.

9) Run the microplate reader and conduct measurement at 450nm.

10) Data analysis: Four parameter curve fitting is recommended.

10. NOTES

1) **Sample Preparation**

a) After collection, the samples should be aliquoted and stored at -20℃ (less than 3 months) or -80℃ (less than 6 months) to maintain protein activity, avoid contamination and multiple freeze-thaw cycles. If samples are to be run within 24 hours, they may be stored at 2- 8℃.

b) Samples should be frozen and aliquoted if not analyzed shortly after collection, avoid multiple freeze-thaw cycles. Thaw the frozen samples completely and mix well by pipettor or Vortex, centrifuge to remove flocculent insoluble substances before use.

c) It is recommended that all standards and samples be run in duplicate. Please gently mix the liquid after adding standards and samples, and confirm there's no bubble inside.

d) The process of protein purification is often accompanied by complex buffer solutions. To exclude matrix effect, it is recommended to perform spike recovery test when using different buffers for the first time. High salt, low PH, polysaccharide, organic solvents, and detergents can result in lower recovery rates. The common practice is to add Standard S1(100ng/ml)/Diluent Buffer into a buffer solution in a 1:4 volume ratio(e.g. add 20ul of standard S1(100ng/ml)/Diluent Buffer to 80ul of the buffer solution). The recovery rate is calculated by subtracting the background concentration without the S1 from the concentration after with S1, then dividing by the theoretical concentration.

2) Experimental Operation

a) Do not mix or interchange different ELISA kits or same ELISA kit but with different lot.

b) To avoid cross-contamination,use disposable sterilized tips during the experiment.

c) Total dispensing time for addition of reagents or samples to the plate should not exceed 10 minutes. If there are too many samples, a multichannel pipettor is recommended.

d) Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by

efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells. Do not directly touch the bottom of the microplate. If it is covered with fingerprints or stains, it will affect the measurement.

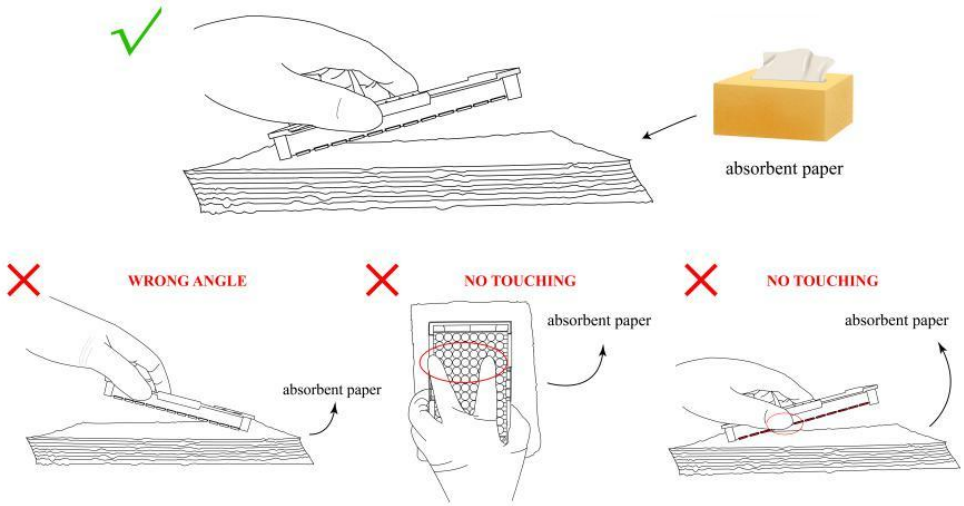
e) TMB is easily contaminated. Please protect it from light and metal.

f) Monitor the reaction time. After adding TMB substrate, observe the color change of reaction wells at regular intervals (for example, about 10 minutes). If the color turns too deep, add Stop Solution in advance to terminate the reaction.

g) The Stop Solution provided with this kit is diluted sulfuric acid. Protective gloves, clothes, and face protection should be worn.

h) The coefficient of determination of the standard curve should be $R^2 \geqslant 0.95$.

i) Schematic diagram of tapping the plate:



11. CALCULATION OF RESULTS

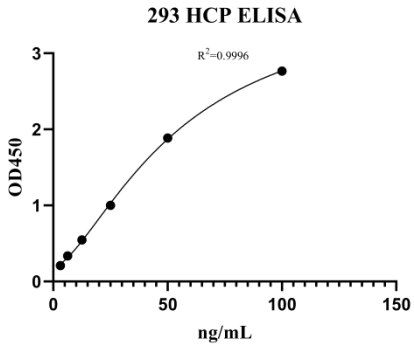
1) Average the duplicate readings for each standard and sample.

2) Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and generate a four paramater logistic (4-PL) curve-fit.

3) Calculate the concentration of samples corresponding to the mean absorbance from the standard curve.

4) Standard curve is for demonstration only.

APPENDIX 1: EXAMPLE OF STANDARD CURVE



ng/ml	OD450
100	2.7655
50	1.885
25	1.0005
12.5	0.547
6.25	0.336
3.125	0.208

12. QUALITY CONTROL

1) Intra Variation%: 7.1-10.6

2) Inter Variation%:6.8-10.4

3) Linearity:

Diluent ratio	Range %
1:2	100.1-101
1:4	96.7-98.8
1:8	99.1-108.2
1:16	93.6-113.5
1:32	82.4-97.7

4) Sensitivity:

LOD: 0.78ng/ml

LOQ: 3.1 ng/ml

293 细胞宿主蛋白（HCP）残留检测试剂盒说明书

规格：48T

应用：定量检测细胞培养上清、蛋白纯化过程及终产物中宿主细胞蛋白残留

本试剂盒仅供科研和生产使用，不得用于临床及诊断！

一、背景介绍

本试剂盒是用来定量分析以 293 细胞为表达系统时，蛋白药物中宿主细胞残留蛋白。

293 细胞系被广泛用来表达治疗性蛋白，在表达目的蛋白的同时，会伴随着表达细胞自身凋亡，资料显示细胞破碎后释放到培养基中的宿主蛋白达上千种之多，其中很大一部分具有很强的免疫原性，导致不良的毒性或免疫反应而危及产品安全和质量，造成潜在的生物污染，生物医药产品生产下游过程的目的之一就是移除这些潜在危害。

因此，非常有必要将宿主细胞蛋白（HCP）残留量降低到最低水平，在研发下游纯化的工艺时，必须具有一种科学合理的测定成品或者半成品中 HCP 浓度的方法，而酶联免疫法具有极高灵敏度，因而被监管机构定为 HCP 检测的金标准。

二、实验原理

本试剂盒采用了固相夹心法的酶联免疫吸附实验（ELISA）。先将捕获抗体包被微孔板，制成固相抗体。检测时在包被抗体的微孔板中先加入待测抗原孵育，洗涤后加入辣根过氧化物酶（HRP）标记的检测抗体，形成包被抗体-抗原-检测抗体复合物。经洗涤后去除未参与反应的结合物，最后加入底物 TMB 显色。TMB 在 HRP 的氧化作用下转化成蓝色，并在酸的作用下转化成最终的黄色。氧化后的 TMB 颜色和因子的总含量呈正相关。用酶标仪在 450nm 波长下测定吸光度（OD 值），与浓度拟合成标准曲线，通过样本 OD 值，代入标准曲线方程，计算样品中因子浓度。

三、试剂盒优势

(1) 覆盖度广：包被抗体及检测抗体均为种属兔来源，有较强的识别 HCP 能力，且该种属个体间差异小，具有高度可比性，工艺稳定。

(2) 抗体滴度高:试剂盒中使用的抗体,在动物免疫阶段采用间接法 Elisa 检测效价达 10^7 以上。

(3) 灵敏度高：血清抗体纯化采用亲和纯化，最大限度去除非特异性抗体。

(4) 稳定性高：生产过程采用广谱蛋白稳定剂，和微孔板处理工艺，增加标准品及微孔板热稳定性和结果的可重复性。

(5) 稀释液优化：使用优化稀释液，可降低样本检测过程中非特异性吸附，本底显色极低利于观察待测样本浓度。

四、实验材料及仪器准备

1. 试剂盒内容（2-8℃冷藏）

	名称	规格	数量	保存
1	已包被平底微孔板	48 孔	1 板（可拆卸）	2-8℃密封冷藏
2	标准品母液（500ug/ml）	30ul	1 管	2-8℃冷藏
3	检测抗体母液(100×)	100ul	1 管	2-8℃冷藏
4	TMB	6ml	1 瓶	2-8℃避光冷藏
5	终止液	6ml	1 瓶	2-8℃冷藏
6	洗液（100×）	10ml	1 瓶	2-8℃冷藏
7	稀释液（10X）	10ml	1 瓶	2-8℃冷藏
8	封板膜		4 张	
9	使用说明书		1 份	

五、实验需要但试剂盒未提供的材料

1) 10-1000μl 移液器及一次性灭菌吸头

2) 多道移液器

3) 灭菌的去离子水或超纯水 1L

4) 灭菌 EP 管

5) 吸水纸

6) 酶标仪

7) 高速离心机

8) 洗板机或者洗瓶

9) 数据分析及绘图软件

六、样本收集

细胞上清液：收集培养的细胞，1000×g（或 3000rpm）离心 15 分钟，取上清，-20℃或-80℃分装保存备用。

样本准备注意事项：

- 样本收集完毕后，要分装保存在-20°C (少于 3 个月) 或-80°C (少于 6 个月)以保持蛋白活性和避免污染。避免反复冻融，如果要在 24 小时内分析样本，可以保存在 2- 8°C。
- 某些化学裂解液可能会对本实验造成干扰，比如 SDS，Triton，谨慎使用。
- 样本液中含有沉淀物会对 ELISA 有干扰，务必离心去除。
- 不能加热来融化样本。

七、实验前的准备

请仔细阅读试剂盒说明书，反应在室温下进行。

八、试剂的准备

1) 试剂盒内所有试剂及包被板，请在使用前的 30min 拿出，使其恢复室温。

2) 洗液的稀释：将 10ml 100×洗液母液，加入 990ml 去离子水或超纯水，混匀备用。如果浓缩液中有少许结晶，请将其置于室温，并轻轻震荡至晶体完全溶解。

3) 稀释液的准备：将 10ml 10×稀释液母液，加入 90ml 去离子水或超纯水，混匀备用。如果浓缩液中有少许结晶，请将其置于室温，并轻轻震荡至晶体完全溶解，用作标准品，样品，检测抗体的稀释液。

九、操作步骤

1. 撕开包装袋，取出包被有抗体的酶标板，拆下不需要使用的板条，并用封口膜封好，放回铝箔袋，重新放回 4℃保存（板架可重复使用）。标准品由于运输颠簸可能会粘在管壁上，使用前轻微甩匀，或在离心机上离心 2 秒左右。

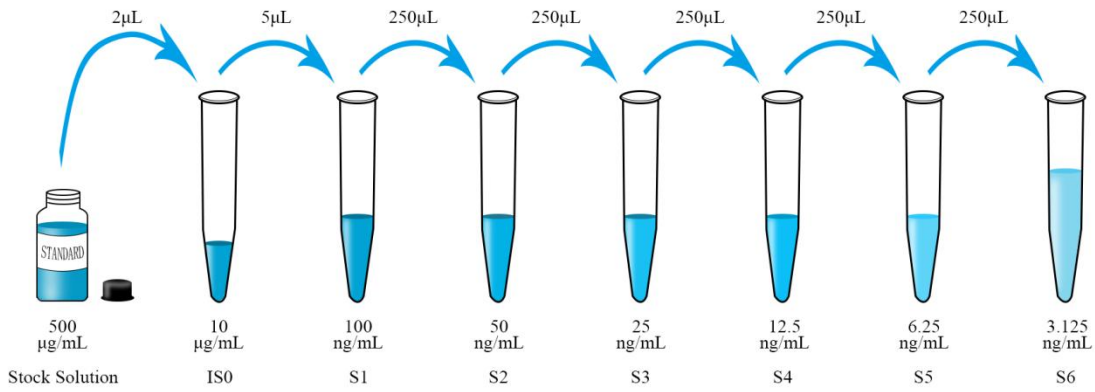
2. 标准品的稀释：

配置标曲时提前标记 7 只样品稀释管，预加入一定体积的稀释液，其中 IS0（98ul）,S1(495ul),S2 至 S6(各 250ul)

取标准品母液（浓度为 500ug/ml）2ul 加入到标记为 IS0 的管，震荡混匀后，接着吸取 5ul 浓度为 10ug/ml 的 IS0 到 S1 管，混匀后，取 250ul 浓度为 100ng/ml 的标准品 S1 到下一管，之后以 2 倍梯度稀释至 S6（稀释过程如下图）。

从稀释好后的标准曲线各浓度点分别取 100ul 加入空白微孔中，样本（原液或稀释液）取 100ul 加入空白微孔中。空白对照(Blank Control)加入 100ul 的样本稀释液即可。

注：标准曲线有 6 个点，分别命名为 S1、S2、S3……S6。其中 S1 即标准曲线的最高浓度点（100ng/ml）。



3. 将酶标板用封板膜密封后室温振荡孵育 1.5h。

4. 洗板机洗板：

- 取出稀释好的洗液放置于洗板机的洗瓶中备用。
- 取出上步中的微孔，甩去微孔中的液体，洗板机洗板5次。

- 洗板完成后，将微孔板倒扣在吸水纸上拍打，充分拍干至无明显水膜为止。

或手动洗板：

- 取出稀释好的洗液放置于洗瓶中备用。
- 取出上步中的微孔，甩掉微孔中的液体，在吸水纸上轻轻拍打至无明显液滴。
- 用多道移液枪向每个微孔中加300ul洗液，静置20s，倒去洗液，将微孔板倒扣在吸水纸上轻轻拍打。重复5次。注：第五次洗板时，充分拍干至无明显水膜为止。

5. 检测抗体：取检测抗体母液(100×)100ul 到 9.9ml 稀释液中稀释至工作浓度(1×)，取 100ul 加入到各微孔中，将酶标板用封板膜密封后室温振荡孵育 1.5h。
6. 洗板：重复步骤 4。
7. 显色：各微孔板加入 100ul TMB 溶液，室温反应 15min 左右。若颜色浅可适当延长反应时间，勿超过 30min。
8. 终止：各微孔中加入 50ul 终止液，终止反应。
9. 读取 OD 值：在波长 450nm 下读取 OD 值。
10. 数据分析：推荐使用四参数回归拟合。

十、注意事项

（一）样本收集注意事项

1、样本收集完毕后，要分装保存在-20℃（少于 3 个月）或-80℃（少于 6 个月）以保持蛋白活性，避免污染和反复冻融。如果要在 24 小时内分析样本，可以保存在 2-8℃。

2、采集样本后如果短期内不使用，请将样本分装后冷冻保存，避免反复冻融。冷冻样本使用前请保证充分化冻，使用前用移液器或者 Vortex 混匀，可离心除去絮状不溶物。

3、建议所有标准品及样本都设置复孔。向微孔中加入试剂或样本后，请轻轻震荡使液体混匀，并尽量保证不要有气泡。

4、目标蛋白纯化过程通常伴随成分复杂的缓冲液，建议首次使用不同缓冲液时进行加标回收，

以排除基质干扰效应。通常，高盐、低pH、多糖、有机溶剂及去污剂会导致较低回收率。

通常做法是，将稀释后的标准品S1（100ng/ml）与待测试溶液基质按照1：4体积混合（如20ul含/不含浓度为100ng/ml的标准品S1加入80ul待测溶液），计算时用加标后浓度减去加标前本底浓度，再除以理论浓度即为加标回收率。

（二）实验操作注意事项

1、请不要将本试剂盒的试剂与其他试剂盒试剂交叉使用。

2、实验操作使用一次性吸头，避免交叉污染。

3、加样：加样时要控制时间和速度，一般加样时间控制在10分钟内。如果样本数量过多，可使用多道移液器。

4、洗涤：洗涤时微孔中残留的洗涤液应在吸水纸上充分拍干，并要消除板底残留的液体和手指痕迹，避免影响最后的酶标仪读数。

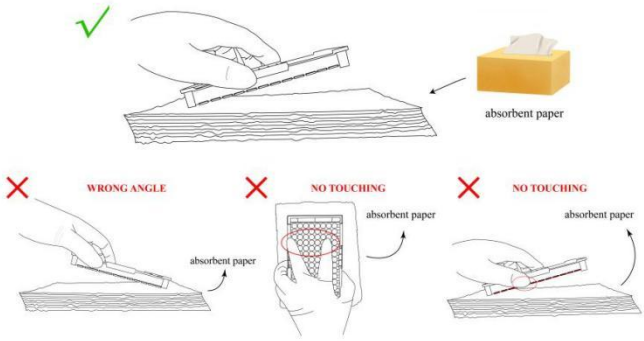
5、由于底物TMB溶液是光感性的试剂，使用前请勿长时间暴露于可见光下。同时要避免TMB与金属接触。

6、反应时间的控制：加入底物后请定时观察反应孔的颜色变化（比如，10分钟左右），如果颜色较深，请提前加入终止液终止反应。

7、本试剂盒中使用了稀硫酸作为终止液，其具有轻微腐蚀性，使用时应避免接触衣物或眼、手等皮肤暴露部位。

8、标准曲线的$R^2 \geqslant 0.95$。

9、拍板示意图:



十一、数据处理

1) 对样本及标准品各自对应的复孔OD值取平均值。

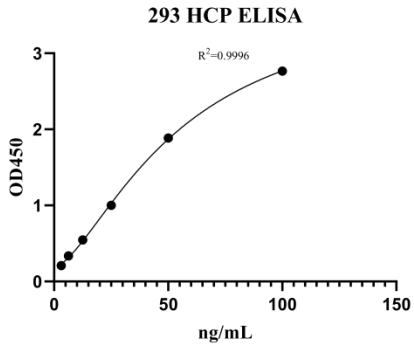
2) 以标准品的 OD 作为 Y 值，标准品的浓度作为 X 值，推荐选择四参数 logistic (4-PL) 曲线拟合。

3) 将样本 OD 代入到标准曲线方程中计算样本中待检样本的浓度。

4) 以下曲线仅供参考。

附件一、标准曲线实例

HCP 标准曲线



ng/ml	OD450
100	2.7655
50	1.885
25	1.0005
12.5	0.547
6.25	0.336
3.125	0.208

十二、试剂盒质量控制

1）批内差CV%: 7.1-10.6

2）批间差CV%: 6.8-10.4

3）线性:

稀释倍数	Range %
1:2	100. 1-101
1:4	96. 7-98. 8
1:8	99. 1-108. 2
1:16	93. 6-113. 5
1:32	82. 4-97. 7

4）灵敏度:

最低检测限(LOD)：0.78ng/ml　　最低定量限(LOQ)：3.1ng/ml