（本试剂盒仅供体外研究使用，不用于临床诊断！）

产品货号：E-EL-E607

产品规格：96T/48T/24T/96T*5

Elabscience® SARS-CoV-2 Spike 蛋白总抗体酶联免疫吸附测定试剂盒使用说明书

SARS-CoV-2 Spike Protein Total Antibody ELISA Kit

使用前请仔细阅读说明书。如果有任何问题，请通过以下方式联系我们：

电话：400-999-2100

邮箱：techsupport@elabscience.cn

网址：www.elabscience.cn

具体保质期请见试剂盒外包装标签。请在保质期内使用试剂盒。

联系时请提供产品批号(见试剂盒标签)，以便我们更高效地为您服务。

用途

该试剂盒用于体外定性检测血清和血浆中抗SARS-CoV-2 Spike蛋白总抗体。

检测原理

本试剂盒采用双抗原夹心 ELISA 法。用 SARS-CoV-2 Spike 蛋白包被于酶标板上,实验时样品(或质控品)中抗 SARS-CoV-2 Spik 蛋白总抗体与包被 SARS-CoV-2 Spike 蛋白结合。游离的成分被洗去。后加入辣根过氧化物酶标记的 SARS-CoV-2 Spike 蛋白，形成抗原-抗体-HRP 标记抗原的免疫复合物。游离的成分被洗去。加入显色底物(TMB)，TMB 在辣根过氧化物酶的催化下呈现蓝色，加终止液后变成黄色。用酶标仪在 450nm 波长处测 OD 值，通过计算抑制率来定性判断抗 SARS-CoV-2 Spike 蛋白总抗体是否在检测样本中存在。

试剂盒组成及保存

未拆封的试剂盒可在 2-8℃保存 12 个月。试剂盒组分开封后请按照下表中的条件分别保存。

中文名称	规格	开封后保存条件
ELISA 酶板 Micro ELISA Plate	96T：8 孔×12 条 48T：8 孔×6 条 24T：8 孔×3 条 96T*5：5 块 96T 酶标板	2-8℃，可存放至有效期(12 个月)
阳性质控 Positive Control	96T：2 支 48T/24T：1 支 96T*5：10 支	
阴性质控 Negative Control	96T：2 支 48T/24T：1 支 96T*5：10 支	
浓缩 HRP 酶结合物 (100×) Concentrated HRP Conjugate (100×)	96T：1 支 120μL 48T/24T：1 支 60μL 96T*5：5 支 120μL	
样本&质控稀释液 Sample & Control Diluent	96T/48T/24T：1 瓶 20mL 96T*5：5 瓶 20mL	2-8℃，可存放至有效期(12 个月)
酶结合物稀释液 HRP Conjugate Diluent	96T/48T/24T：1 瓶 14mL 96T*5：5 瓶 14mL	
浓缩洗涤液（25×） Concentrated Wash Buffer (25×)	96T/48T/24T：1 瓶 30mL 96T*5：5 瓶 30mL	
底物溶液(TMB) Substrate Reagent	96T/48T/24T：1 瓶 10mL 96T*5：5 瓶 10mL	
反应终止液 Stop Solution	96T/48T/24T：1 瓶 10mL 96T*5：5 瓶 10mL	
封板覆膜 Plate Sealer	96T/48T/24T：5 张 96T*5：25 张	
产品说明书 Product Description	1 份	
质检报告 Certificate of Analysis	1 份	

说明：**浓缩HRP酶结合物(100×)**和**底物溶液(TMB)**请避光保存。

所有试剂瓶盖须旋紧以防止蒸发和微生物的污染。

试剂体积以实际发货版说明书为准。相关试剂在分装时会比标签上标明的体积稍多一些，请在使用时量取而非直接倒出。

试验所需自备物品

1. 酶标仪(450 nm波长滤光片)
2. 高精度移液器，EP管及一次性吸头：0.5-10μL, 2-20μL, 20-200μL, 200-1000μL
3. 37℃恒温箱，
4. 双蒸水或去离子水
5. 吸水纸
6. 加样槽

样品收集方法

(具体处理方法可参考官网：http://www.elabscience.cn/List-detail-241.html)

1. **血清**：全血样品于室温放置1小时或2-8℃过夜后于2-8℃，1000×g离心20分钟，取上清即可检测。
2. **血浆**：抗凝剂推荐使用EDTA-Na_2，样品采集后30分钟内于2-8℃，1000×g离心15分钟，取上清即可检测。

注意事项

■ 试剂盒注意事项

1) 本试剂盒仅供体外研究使用。

2) 试验中请穿着实验服并戴乳胶手套做好防护工作。特别是检测血液或者其他体液样品时，请按国家生物试验室安全防护条例执行。

3) 刚开启的酶标板孔中可能会有少许水样物质，此为正常现象，不会对实验结果造成任何影响。暂时不用的板条应拆卸后放入备用铝箔袋，按照上述表格中保存条件存放。

4) 检测使用的酶标仪需要安装能检测450±2 nm波长的滤光片，光密度范围在0-3.5之间。建议使用时提前15分钟预热。

5) 请勿使用其他批号或其他来源的试剂混合或替代本试剂盒中的试剂。

6) 试验中所用的EP管和吸头均为一次性使用，严禁混用。

7) 请勿使用过期的试剂。

■ 样品注意事项

1) 收集血液的试管应为一次性无内毒素试管。避免使用溶血，高血脂样品。

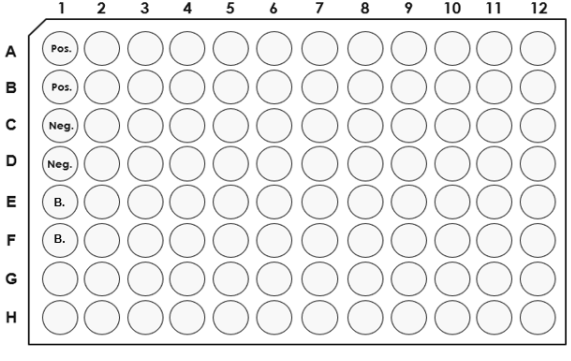
2) 谨慎处理血清血浆样本。

3) 样品收集后若在3天内进行检测可保存于2-8℃，若不能及时检测，请按一次使用量分装，冻存于-20℃(1个月内检测)，或-80℃(3个月内检测)，避免反复冻融。在检测前，冷冻过的样本应缓慢地融化并离心除去冻融过程产生的沉淀物。样本需恢复至室温后混匀使用。

检测前准备工作

1. 提前20分钟从冰箱中取出试剂盒，平衡至室温(18-25℃)。如果试剂盒需分多次使用，请仅取出本次实验所需的酶标板条和试剂，剩余板条和试剂需按照指定条件保存。
2. **洗涤液：**将**浓缩洗涤液**用双蒸水稀释(1:24)。提示：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象，可用40℃水浴微加热使结晶完全溶解后再配制洗涤液。当日使用。
3. **HRP酶结合Spike蛋白工作液：**HRP酶结合物为HRP酶结合Spike蛋白。实验前计算当次实验所需用量(以100 μL/孔计算)，实际配制时应多配制100-200 μL。使用前15分钟，将**浓缩HRP酶结物**于800×g离心1分钟，以**酶结合物稀释液**将100×**浓缩HRP酶结合物**稀释成1×工作浓度(例如：10 μL浓缩液+990 μL稀释液)。HRP酶结合物工作液需要在2-8℃保存并在1天内使用。
4. **样品：**使用样品&质控品稀释液将样品稀释至少10倍。
5. **阳性/阴性质控品：**将质控品于10000×g离心1分钟，加入**标准品&质控品稀释液**0.5 mL，旋紧管盖，静置10分钟，上下颠倒数次，待其充分溶解后，轻轻混匀，避免起泡。
6. 预处理过的样品和质控品均需要在2-8℃保存并在1天内使用。

上样程序



Pos.：阳性质控品; **Neg.**：阴性质控品; **B.**：空白对照；

操作步骤

1. 分别设定**样品孔、阳性质控孔、阴性质控孔和空白对照孔**(仅加入底物和终止液)。加入 100 μL 预处理过的样品和质控品后(建议样品和质控品在检测中均设立复孔)，给酶标板覆膜，37℃孵育 90 分钟。提示：加样时将样品加于酶标板底部，尽量不触及孔壁，轻轻晃动混匀，避免产生气泡。加样时间宜控制在 10 分钟内。
2. 甩尽孔内液体，在洁净的吸水纸上拍干。每孔加**洗涤液** 350 μL，浸泡 1 分钟，吸去或甩掉酶标板内的液体，拍干。重复此洗板步骤 3 次。提示：此处可使用洗板机(参考北京拓普 DEM-3 型洗板机参数设置：2 点吸，每孔加入洗涤液 350 μL，振板 5 秒，吸液 0.5 秒)。洗板完成后请立即进行下步操作，不要让微孔板干燥。
3. 每孔加**酶结合物工作液** 100μL(空白对照孔不加)，酶标板加上覆膜，37℃温育 30 分钟。
4. 每孔加**底物溶液(TMB)** 90 μL，酶标板加上覆膜，37℃避光孵育 15 分钟左右。提示：根据实际显色情况酌情缩短或延长，但不可超过 30 分钟。
5. 每孔加**终止液** 50 μL，终止反应。提示：终止液的加入顺序应尽量与底物溶液的加入顺序相同。
6. 立即用酶标仪在 450 nm 波长测量各孔的光密度(OD 值)。

操作一览表

对应板孔中加入 100μL 预处理的样品，阳性质控品和阴性质控品

↓ 37℃, 90 min

弃掉板孔液体，洗板 3 次

↓

每孔加入 100μL HRP 酶结合物工作液

↓ 37℃, 30 min

弃掉板孔液体，洗板 5 次

↓

每孔加入 90μL 底物溶液

↓ 37℃, 15 min

每孔加入 50μL 终止液

↓

立即在450nm波长下读数，处理数据

结果计算

1. **计算方法**
   (1) 每次实验都需根据 Cut Off 值单独判断结果。
   (2) Cut Off 值= 阴性质控平均吸光度 A450 ×2.1.。
2. **质量控制**
   (1) 空白孔(实验时只加入底物溶液和终止液) 吸光度 A450≤0.08。
   (2) 阳性质控吸光度 A450＞ 1.0。
   (3) 阴性质控吸光度 A450≤0.18。

注意：只有当质量控制符合要求时，实验结果才可靠！

3. **结果判断**
   (1) 阳性结果：样本吸光度≥Cut Off 值
   检测的样本被认为是抗 SARS-CoV-2 Spike 蛋白总抗体阳性。
   (2) 阴性结果：样本吸光度＜Cut Off 值
   检测的样本被认为是抗 SARS-CoV-2 Spike 蛋白总抗体阴性。

产品性能

批内CV：CV%＜8%
高中低浓度3个样本使用同一酶标板重复验证20次，计算板内精密性。
批间CV：CV%＜10%
高中低浓度3个样本在3个不同批次的酶标板上重复验证20次，计算批间精密性。

声明

1. 限于现有条件及科学技术水平，尚不能对所有原料进行全面的鉴定分析，本产品可能存在一定的质量技术风险。
2. 本试剂盒在研发过程中去除/降低了生物学样本中的一些内源性干扰因素，并非所有可能影响的因素均已去除。
3. 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等因素密切相关，本公司只对试剂盒本身负责，不对因使用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可能的使用量，预留充足的样本。
4. 为了达到好的实验结果，请只使用本公司试剂盒内提供的试剂，不要混用其他制造商的产品，严格按照说明书操作。
5. 由于操作过程中试剂制备以及酶标仪参数设置不正确，可能导致结果异常，实验前请仔细阅读说明书并调整好仪器。
6. 即使是相同人员操作也可能在两次独立实验中得到不同的结果，为保证结果的重现性，需要控制实验过程中每一步的操作。
7. 试剂盒发货前会经过严格的质检，然而，因为运输条件、实验设备差异等等因素影响，用户检测结果可能跟出厂数据不一致。不同批次间试剂盒间的差异也可能来自上述原因。
8. 本试剂盒未与其他厂家同类试剂盒或不同方法检测同一目的物的产品进行对比，所以不排除检测结果不一致的情况。
9. 试剂盒仅供研究使用，如将其用于临床诊断或任何其他用途，我公司将不对因此产生的问题负责，亦不承担任何法律责任。

SARS-CoV-2 Spike Protein Total Antibody ELISA Kit

Catalog No: E-EL-E607
Size: 96T/48T/24T/96T*5

Intended use

This ELISA kit applies to the in vitro qualitative determination of total antibodies against SARS-CoV-2 Spike Protein in serum or plasma.

Test principle

This ELISA kit uses the Sandwich-Ag ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with recombinant SARS-CoV-2 Spike Protein. After adding samples (or controls) to wells, the total antibodies against SARS-CoV-2 Spike Protein will combine with the pre-coated SARS-CoV-2 Spike Protein. After washing completely, add Horseradish Peroxidase (HRP) conjugated SARS-CoV-2 Spike Protein to develop the antigen-antibody-HRP conjugated antigen complex. Free components are washed away. The substrate solution is added to each well. Only those wells that contain SARS-CoV-2 Spike Protein, antibodies against SARS-CoV-2 Spike Protein and HRP conjugated SARS-CoV-2 Spike Protein will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ±2 nm. Compared with the CUT OFF value to judge whether SARS-CoV-2 Spike Protein total antibody exists in the tested samples or not.

Other supplies required

Microplate reader with 450 nm wavelength filter
High-precision transfer pipette, EP tubes and disposable pipette tips
Incubator capable of maintaining 37℃
Deionized or distilled water
Absorbent paper
Loading slot

Kit components & Storage

An unopened kit can be stored at 2-8℃ for 12 months. After opening, store the items separately according to the following conditions.

Item	Specifications	Storage
Micro ELISA Plate (Dismountable)	24T: 8 wells ×3 strips 48T: 8 wells ×6 strips 96T: 8 wells ×12 strips 96T*5: 5 plates, 96T	2-8℃, up to expiry date (12 months)
Positive Control	24T: 1 vial 48T: 1 vial 96T: 2 vials 96T*5: 10 vials	
Negative Control	24T: 1 vial 48T: 1 vial 96T: 2 vials 96T*5: 10 vials	
Concentrated HRP Conjugate (100×)	96T: 1 vial, 120 µL 48T/24T: 1 vial, 60 µL 96T*5: 5 vials, 120 µL	
Sample & Control Diluent	96T/48T/24T: 1 vial, 20 mL 96T*5: 5 vials, 20 mL	2-8℃, up to expiry date (12 months)
HRP Conjugate Diluent	96T/48T/24T: 1 vial, 14 mL 96T*5: 5 vials, 14 mL	
Concentrated Wash Buffer(25×)	96T/48T /24T: 1 vial, 30 mL 96T*5: 5 vials, 30 mL	
Substrate Reagent	96T/48T /24T: 1 vial, 10 mL 96T*5: 5 vials, 10 mL	
Stop Solution	96T/48T /24T: 1 vial, 10 mL 96T*5: 5 vials, 10 mL	
Plate Sealer	96T/48T /24T: 5 pieces 96T*5: 25 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Note: Concentrated HRP Conjugate(100×) and **Substrate Reagent** should be stored away from light.

All reagent bottle caps must be tightened to prevent evaporation and microbial pollution. The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

Sample collection

(More detailed information please view our website: https://www.elabscience.com/List-detail-259.html)

Serum: Allow samples to clot for 1 hour at room temperature or overnight at 2-8℃before centrifugation for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

Plasma: Collect plasma using EDTA-Na_2 as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8℃ within 30 min of collection. Collect the supernatant to carry out the assay.

Note

■ **Note for kit**

1) For research use only.
2) Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
3) A freshly opened ELISA plate may appear a water-like substance, which is normal and will not have any impact on the experimental results. Return the unused wells to the foil pouch and store according to the conditions suggested in the above table.
4) The microplate reader should be able to be installed with a filter that can detect the wave length at 450 ±2 nm. The optical density should be within 0-3.5. Follow the Instructions of the Microplate Reader for set-up and preheat it for 15 min before OD measurement.
5) Do not mix or substitute reagents with those from other lots or sources.
6) Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.
7) The kit should not be used beyond the expiration date on the kit label.

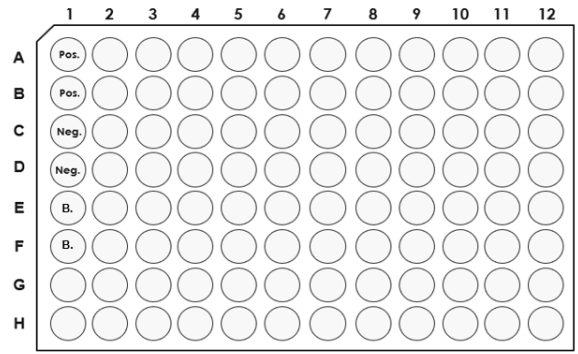
■ **Note for sample**

1) Tubes for blood collection should be disposable and be non-endotoxin. Severe hemolysis, lipoid, or turbidity samples should not be used.
2) Handle all serum and plasma as if capable of transmitting infectious agents.
3) Samples should be assayed within 3 days when stored at 2-8℃, otherwise samples must be divided up and stored at -20℃ (≤ 1 month) or -80℃ (≤ 3 months). Avoid repeated freeze-thaw cycles. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates. Frozen samples must be mixed well and brought to room temperature before testing.

Reagent preparation

1. Bring all reagents to room temperature (18-25℃) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
2. **Wash Buffer:** Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40℃ water bath and mix it gently until the crystals have completely dissolved.
3. **HRP Conjugated working solution:** HRP Conjugate is HRP Conjugated Spike Protein. Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate Spike Protein at 800×g for 1 min, then dilute the 100×**Concentrated HRP Conjugate** to 1× working solution with **HRP Conjugated Diluent** (Concentrated HRP Conjugate: HRP Conjugated Diluent= 1: 99). Note: The HRP Conjugate working solution should be stored at 2-8℃ and used within 1 day.
4. **Samples**: Dilute the tested serum or plasma at least 10 fold by using the **Sample & Control Diluent**, mix thoroughly.
5. **Positive Control/ Negative Control working solution**: Centrifuge the Controls at 10,000×g for 1 min. Add 0.5 mL of Sample & Control Diluent, let it stand for 10 min and invert it gently several times.
6. Sample and Control working solution should be stored at 2-8℃ and used within 1 day.

Pipetting protocol

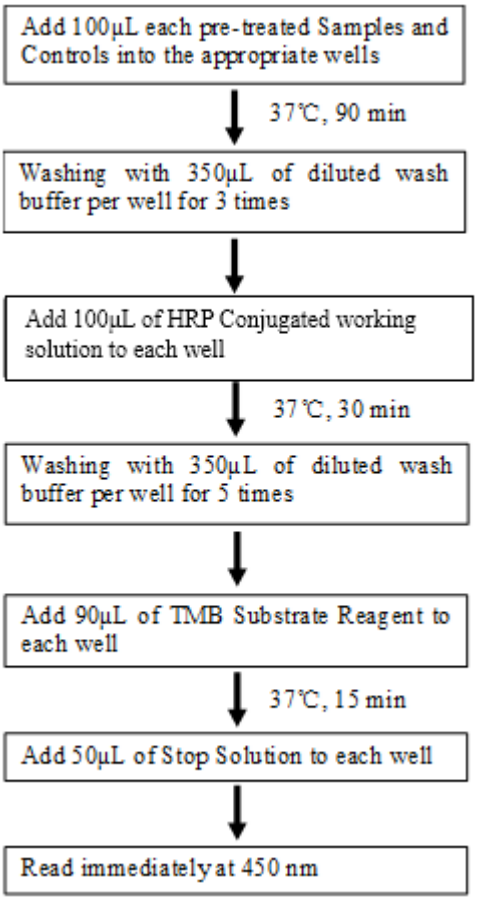


Pos.: Positive Control; **Neg.**: Negative Control; **B.**: Blank;

Assay procedure

1. Determine wells for **Positive Control**, **Negative Control, Blank** (Do not add any reagents except Substrate Reagent and Stop Solution) and **Sample**. Add 100μL of pre-treated Samples and Controls into the appropriate wells (It is recommended that all samples, Controls and blank be assayed in duplicate). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the bottom of the micro TEST plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Decant the solution from each well, add 350μL of **wash buffer** to each well. Soak for 1-2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.
3. Add 100μL of **HRP Conjugated working solution** to each well (except the blank well). Cover with the Plate sealer. Incubate for 30 min at 37℃.
4. Decant the solution from each well, repeat the wash process for 5 times as conducted in step 2.
5. Add 90μL of **Substrate Reagent** to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37℃. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min. Preheat the Microplate Reader for about 15 min before OD measurement.
6. Add 50μL of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
7. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Assay Procedure Summary



Calculation

4. **Calculation method**
   (1) Use each assay result independently, determination of result according to Cut Off value.
   (2) Calculate the Cut Off: Cut Off = Negative Control average A_{450} × 2.1.
5. **Quality control**
   (1) Blank well (add substrate reagent and stop solution only) absorbance ≤ 0.08.
   (2) Positive Control A_{450} ＞1.0.
   (3) Negative Control A_{450} ≤ 0.18.

   Note: Experimental result is valid if any quality control is valid.
6. **Judgment method**
   (1) Positive result: Sample absorbance ≥ Cut Off
   The tested sample is classified as positive for total antibodies against SARS-CoV-2 Spike Protein.
   (2) Negative result: Sample absorbance ＜Cut Off
   The tested sample is classified as negative for total antibodies against SARS-CoV-2 Spike Protein.

Performance

Intra-CV: CV%＜8%

3 samples with low, mid-range and high level were tested 20 times on one plate respectively.

Inter-CV: CV%＜10%

3 samples with low, mid-range and high level were tested on 3 different plates, 20 replicates in each plate.

Declaration

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. 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.
3. The final experimental results will be closely related to the validity of products, operational skills of the operators, the experimental environments and so on. We are only responsible for the kit itself, but not for the samples consumed during the assay. The users should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
4. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions.
5. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
6. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
7. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipment, and so on. Intra-assay variance among kits from different batches might arise from the above reasons, too.
8. Kits from different manufacturers or other methods for testing the same analyte could bring out inconsistent results, since we haven’t compared our products with those from other manufacturers.
9. The kit is designed for research use only, we will not be responsible for any issues if the kit is applied in clinical diagnosis or any other related procedures.