（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-E608

产品规格：24T/48T/96T/96T*5

# Elabscience® SARS-CoV-2 中和抗体酶联免疫吸附测定试剂盒使用说明书

SARS-CoV-2 Neutralization 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
该试剂盒用于体外半定量检测人血清和血浆中抗SARS-CoV-2中和抗体。

## 基本性能

| 性能 | |
|---|---|
| 灵敏度 | 9.38 ng/mL |
| 检测范围 | 15.63-500 ng/mL |
| 特异性 | 可检测样本中的人 SARS-CoV-2 中和抗体，且与其它类似物无明显交叉反应 |
| 重复性 | 板内，板间变异系数均<10% |

## 检测原理
本试剂盒采用竞争 ELISA 法。用人 ACE2 蛋白包被于酶标板上，实验时样品（或质控品）中的抗 SARS-CoV-2 中和抗体与包被的人 ACE2 竞争辣根过氧化物酶标记的 RBD 的结合位点，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 450 nm 波长处测 OD 值，SARS-CoV-2 中和抗体浓度与 $OD_{450}$ 值之间呈反比，通过绘制标准曲线计算出样品中 SARS-CoV-2 中和抗体的浓度。

## 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

## 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 酶标板<br>Micro ELISA Plate | 24T：8 孔×3 条<br>48T：8 孔×6 条<br>96T：8 孔×12 条<br>96T*5：5 块 96T 酶标板 | 2-8℃，可存放至有效期(12 个月) |
| 冻干标准品<br>Reference Standard | 24T/48T：1 支<br>96T：2 支<br>96T*5：10 支 | |
| 阳性质控品<br>Positive Control | 24T/48T：1 支<br>96T：2 支<br>96T*5：10 支 | |
| 阴性质控品<br>Negative Control | 24T/48T：1 支<br>96T：2 支<br>96T*5：10 支 | |
| 浓缩 HRP 酶结合物（100×）<br>Concentrated HRP Conjugate (100×) | 24T/48T：1 支 60μL<br>96T：1 支 120μL<br>96T*5：5 支 120μL | |
| 标准品&样品稀释液<br>Reference Standard & Sample Diluent | 24T/48T/96T：1 瓶 20mL<br>96T*5：5 瓶 20mL | 2-8℃，可存放至有效期(12 个月) |
| 酶结合物稀释液<br>HRP Conjugated Diluent | 24T/48T 96T：1 瓶 14mL<br>96T*5：5 瓶 14mL | |
| 浓缩洗涤液（25×）<br>Concentrated Wash Buffer (25×) | 24T/48T/96T：1 瓶 30mL<br>96T*5：5 瓶 30mL | |
| 底物溶液(TMB)<br>Substrate Reagent | 24T/48T/96T：1 瓶 10mL<br>96T*5：5 瓶 10mL | |
| 反应终止液<br>Stop Solution | 24T/48T/96T：1 瓶 10mL<br>96T*5：5 瓶 10mL | |
| 封板覆膜<br>Plate Sealer | 24T/48T/96T：5 张<br>96T*5：25 张 | |
| 产品说明书<br>Manual | 1 份 | |
| 质检报告<br>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。

## 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±10 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**

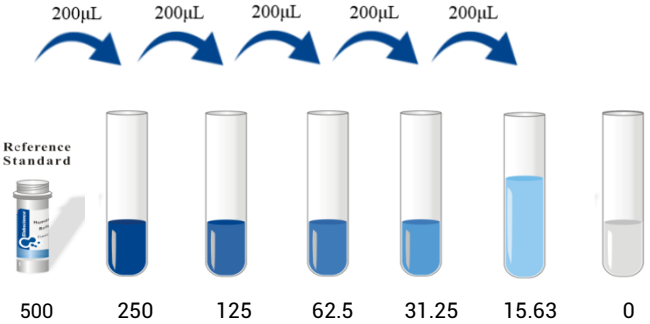
1) 收集血液的试管应为一次性无内毒素试管。避免使用溶血，高血脂样品。
2) 谨慎处理血清血浆样本。
3) 样品收集后若在3天内进行检测可保存于2-8℃，若不能及时检测，请按一次使用量分装，冻存于-20℃(1个月内检测)，或-80℃(3个月内检测)，避免反复冻融。在检测前，冷冻过的样本应缓慢地融化并离心除去冻融过程产生的沉淀物。样本需恢复至室温后混匀使用。

## 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. **标准品工作液：**将**标准品**于10000×g离心1分钟，加入**标准品&样品稀释液** 0.4 mL至冻干标准品中，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，配成500 ng/mL的标准品工作液(或加入0.4 mL**标准品&样品稀释液**后，静置1-2分钟，用低速涡旋仪充分混匀。可通过低速离心去除涡旋过程中产生的气泡)。然后根据需要进行倍比稀释。建议配制成以下浓度：500、250、125、62.5、31.25、15.63、0 ng/mL。

倍比稀释方法：取6支EP管，每管中加入200 µL**标准品&样品稀释液**，从500 ng/mL的标准品工作液中吸取200 µL到第一支EP管中混匀配成250 ng/mL的标准品工作液，按此步骤往后依次吸取混匀。如下图。提示：最后一管直接作为空白孔，不需要再从倒数第二管中吸取液体。

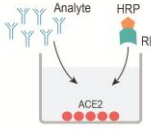



4. **HRP酶结合物工作液：** HRP酶结合物为**HRP酶结合RBD**.实验前计算当次实验所需用量(以50 µL/孔计算)，实际配制时应多配制100-200 µL。使用前15分钟，将**浓缩HRP酶结合物**于800×g离心1分钟，以**酶结合物稀释液**将100×**浓缩HRP酶结合物**稀释成1×工作浓度(例如：10 µL浓缩液+990 µL稀释液)。HRP酶结合物工作液需要在2-8℃保存并在1天内使用。
5. **样品：** 使用**标准品&样品稀释液**将样品稀释10倍。
6. **阳性质控品：** 用0.2 mL**标准品&样品稀释液**溶解阳性质控品。
7. **阴性质控品：** 用0.5 mL**标准品&样品稀释液**溶解阴性质控品。
8. 溶解的标准品和预处理过的样品/质控品均需要在2-8℃保存并在1天内使用。

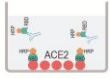
## 操作步骤

1. 分别设定**标准品孔，空白孔，样品孔，阳性质控孔和阴性质控孔**。加入 50 μL 标准品及预处理过的样品和质控品后(建议标准品，样品和质控品在检测中均设立复孔)，立即每孔加入配好的 **HRP 酶结合物工作液** 50 μL。给酶标板覆膜，37℃孵育 60 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**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。
4. 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。
5. 立即用酶标仪在 450 nm 波长测量各孔的光密度(OD 值)。

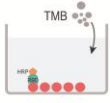
# 操作一览表



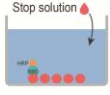

1. 对应板孔内加入 50μL 预处理的标准品，样本和质控品，立即每孔加入50μL HRP酶结合RBD工作液，37℃孵育60分钟




2. 弃掉板内液体，洗板3次




**3.每孔内加入 90μL 底物溶液，37℃孵育15分钟左右**




4.每孔内加入 50μL 终止液

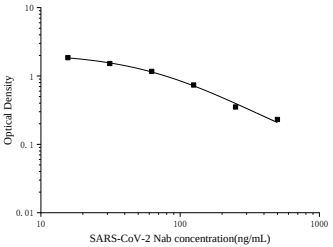
5. 立即在450nm波长下读数，处理数据

## 结果判断

1. 计算标准品和样本复孔的平均OD值，以浓度为横坐标，OD值为纵坐标，在双对数坐标轴上拟合四参数逻辑函数的标准曲线。操作详情请参阅“Elabscience”官网/微信公众号内技术文章。
2. 若样品OD值低于标准曲线下限，应适当稀释后重测并在计算样本浓度时乘以相应的稀释倍数。

## 典型数据

以下数据和曲线仅供参考，实验者需根据自己的实验建立标准曲线。

| ng/mL | OD | 标曲 |
|---|---|---|
| 500 | 0.231 |  |
| 250 | 0.352 | |
| 125 | 0.737 | |
| 62.5 | 1.165 | |
| 31.25 | 1.519 | |
| 15.63 | 1.853 | |
| 0 | 2.260 | |



## 质量控制

每次试验都需要通过质控品的检测浓度值来进行质量控制。
阴性质控品：≤ 15.63 ng/mL
阳性质控品：50-200 ng/mL
若质控品测值不符合上述要求，表示测试无效，建议重复实验。

## 性能

- **精密度**

  板内精密度:低浓度样本,中浓度样本和高浓度样本分别在1块板子上检测20次。板间精密度:低浓度样本,中浓度样本和高浓度样本分别在3块板子上检测20次。

| | 批内变异系数 | | | 批间变异系数 | | |
|---|---|---|---|---|---|---|
| 样本 | 1 | 2 | 3 | 1 | 2 | 3 |
| 数量 | 20 | 20 | 20 | 20 | 20 | 20 |
| 平均值(ng/mL) | 17.74 | 110.98 | 413.33 | 18.21 | 117.00 | 424.56 |
| 标准差 | 0.84 | 7.41 | 34.77 | 1.28 | 9.77 | 28.65 |
| 变异系数（%） | 4.73 | 6.68 | 8.41 | 7.05 | 8.35 | 6.75 |

■ **回收率**

分别往不同样本中添加已知浓度的人 SARS-CoV-2 Nab，做回收实验，得出回收率范围和平均回收率。

| 样本类型 | 回收率范围（%） | 平均回收率（%） |
|---|---|---|
| 血清(n=8) | 90-101 | 94 |
| 血浆(EDTA)(n=8) | 95-107 | 101 |
| 血浆(肝素) (n=8) | 92-106 | 98 |

■ **线性**

将添加有人SARS-CoV-2 Nab的样本分别稀释9倍，18倍，36倍，72倍做回收实验，得出回收率范围及平均回收率。

| 稀释倍数 | | 血清（n=10） | 血浆(EDTA) (n=10) | 血浆(肝素)(n=10) |
|---|---|---|---|---|
| 1:9 | 回收率范围(%) | 78-101 | 89-112 | 79-103 |
| | 平均回收率(%) | 91 | 101 | 92 |
| 1:18 | 回收率范围(%) | 82-97 | 80-101 | 82-115 |
| | 平均回收率(%) | 88 | 91 | 98 |
| 1:36 | 回收率范围(%) | 81-103 | 84-121 | 91-99 |
| | 平均回收率(%) | 91 | 103 | 95 |
| 1:72 | 回收率范围(%) | 79-95 | 91-108 | 86-107 |
| | 平均回收率(%) | 87 | 98 | 96 |

■ **测试数据**

随机挑选121个健康志愿者(未感染过SARS-CoV-2，也未接种过疫苗)和77个疫苗接种者的样本(血清和血浆)进行检测，结果如下:

121 个健康志愿者样本检测数据（OD450)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1.871 | 1.867 | 1.971 | 1.855 | 2.142 | 2.234 | 2.236 | 2.257 | 2.018 | 2.259 | 2.057 |
| 2.225 | 1.904 | 2.062 | 1.836 | 1.84 | 2.204 | 1.856 | 2.04 | 2.274 | 1.913 | 2.184 |
| 2.263 | 2.014 | 1.983 | 1.97 | 1.878 | 2.237 | 2.086 | 1.948 | 2.151 | 1.925 | 2.218 |
| 2.114 | 2.146 | 2.27 | 2.041 | 1.859 | 1.953 | 1.859 | 2.131 | 1.853 | 1.855 | 2.092 |
| 1.893 | 2.215 | 2.199 | 2.252 | 1.934 | 2.052 | 1.834 | 1.825 | 1.897 | 2.206 | 2.088 |
| 1.918 | 2.038 | 2.044 | 2.008 | 2.23 | 1.992 | 2.208 | 1.851 | 2.161 | 2.005 | 1.895 |
| 1.905 | 2.211 | 2.225 | 2.012 | 1.965 | 2.238 | 2.202 | 1.885 | 2.084 | 2.223 | 1.901 |
| 1.886 | 2.255 | 2.151 | 1.955 | 2.001 | 2.172 | 2.193 | 2.108 | 1.823 | 1.938 | 2.009 |
| 2.003 | 1.842 | 1.923 | 2.013 | 2.038 | 1.921 | 1.907 | 1.98 | 2.028 | 1.934 | 2.069 |
| 2.25 | 2.204 | 2.234 | 2.085 | 2.104 | 1.828 | 1.854 | 2.083 | 2.07 | 1.863 | 2.084 |
| 2.246 | 1.838 | 2.047 | 1.898 | 2.044 | 2.251 | 2.099 | 2.05 | 2.124 | 1.838 | 1.975 |

77 个疫苗接种者样本检测数据(OD450)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.815 | 0.573 | 1.175 | 1.234 | 1.552 | 0.682 | 1.148 | 0.647 | 0.944 | 0.443 | 0.945 |
| 1.419 | 0.333 | 0.505 | 0.91 | 1.097 | 0.929 | 0.422 | 1.136 | 0.959 | 1.002 | 0.873 |
| 1.196 | 1.553 | 0.335 | 1.442 | 0.924 | 1.554 | 0.112 | 0.811 | 1.337 | 1.045 | 1.433 |
| 0.907 | 0.999 | 0.773 | 0.531 | 0.65 | 1.195 | 1.532 | 0.943 | 1.015 | 0.261 | 0.113 |
| 0.846 | 0.382 | 0.937 | 1.507 | 1.051 | 1.452 | 0.492 | 0.936 | 0.145 | 0.692 | 0.631 |
| 1.047 | 0.397 | 0.45 | 0.497 | 1.544 | 0.464 | 0.662 | 0.618 | 1.069 | 0.985 | 1.318 |
| 0.497 | 0.115 | 0.307 | 0.318 | 0.951 | 0.349 | 0.377 | 0.627 | 0.194 | 0.333 | 0.185 |

## 问题分析

若实验效果不好，请及时对显色结果拍照，保存实验数据，保留所用板条及未使用试剂，然后联系我公司技术支持为您解决问题。同时您也可以参考以下资料：

| 问题描述 | 可能原因 | 相应对策 |
| --- | --- | --- |
| 标准曲线梯度差 | 吸液或加液不准 | 检查移液器及吸头 |
| | 标准品稀释不正确 | 溶解标准品时稍微旋转瓶身，轻轻混匀使粉末完全溶解 |
| | 洗涤不完全 | 保证洗涤时间和洗涤次数及每孔的加液 |
| 显色很弱或无色 | 孵育时间太短 | 保证充足的孵育时间 |
| | 实验温度不正确 | 使用推荐的实验温度 |
| | 试剂体积不够或漏加 | 检查吸液及加液过程，保证所有试剂按顺序足量添加 |
| | 稀释不正确 | |
| | 酶标记物失活或底物失效 | 混合酶结合物和底物，通过迅速显色来检查判断 |
| 读数数值低 | 酶标仪设置不正确 | 在酶标仪上检查波长及滤光片设置 |
| | | 提前打开酶标仪预热 |
| 变异系数大 | 加液不正确 | 检查加液情况 |
| 背景值高 | 检测抗体的工作浓度过高 | 使用推荐的稀释倍数 |
| | 酶标板洗涤不完全 | 保证每步清洗完全；如果用自动洗板机，请检查所有的出口是否有堵塞；是否使用试剂盒配备的洗涤液 |
| | 洗液有污染 | 配制新鲜的洗液 |
| 灵敏度低 | ELISA 试剂盒保存不当 | 按说明书要求保存相关试剂 |
| | 读数前未终止 | OD 读数前应在每孔中加入终止液 |

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## SARS-CoV-2 Neutralization Antibody ELISA Kit

Catalog No: E-EL-E608
Size: 24T/48T/96T/96T*5

## Intended use

This ELISA kit applies to the in vitro semi-quantitative determination of Neutralization antibodies against SARS-CoV-2 in human serum or plasma.

## Character

| Item | |
|---|---|
| Sensitivity | 9.38 ng/mL |
| Detection Range | 15.63-500 ng/mL |
| Specificity | This kit recognizes SARS-CoV-2 Neutralization Antibody in samples. No significant cross-reactivity or interference between SARS-CoV-2 Neutralization Antibody and analogues was observed |
| Repeatability | Coefficient of variation is < 10% |

## Test principle

This Test kit uses Competitive-ELISA as the method to semi-quantitatively detect the Anti-SARS-CoV-2 Neutralization Antibody in the sample.
The micro ELISA plate provided in this kit is pre-coated with recombinant human ACE2. During the reaction, the SARS-CoV-2 Neutralization Antibody in the pretreated samples or standards/controls competes with a fixed amount of human ACE2 on the solid phase supporter for sites on the Horseradish peroxidase (HRP) conjugated recombinant SARS-CoV-2 RBD fragment (HRP-RBD). After 37℃ incubation, the unbound HRP-RBD as well as any HRP-RBD bound to non-Neutralization antibody will be captured on the plate and eventually form the ACE2-RBD-HRP complex, while the circulating neutralization antibodies HRP-RBD complexes remain in the supernatant and are removed during washing. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of SARS-CoV-2 Neutralization Antibody in the samples is then determined by comparing the OD of the samples to the standard curve.

## 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<br>48T: 8 wells ×6 strips<br>96T: 8 wells ×12 strips<br>96T*5: 5 plates, 96T | 2-8℃, up to expiry date (12 months) |
| Reference Standard | 24T/48T: 1 vial<br>96T: 2 vials<br>96T*5: 10 vials | |
| Positive Control | 24T/48T: 1 vial<br>96T: 2 vials<br>96T*5: 10 vials | |
| Negative Control | 24T/48T: 1 vial<br>96T: 2 vials<br>96T*5: 10 vials | |
| Concentrated HRP Conjugate (100×) | 24T/48T: 1 vial, 60 μL<br>96T: 1 vial, 120 μL<br>96T*5: 5 vials, 120 μL | |
| Reference Standard & Sample Diluent | 24T/48T/96T: 1 vial, 20 mL<br>96T*5: 5 vials, 20 mL | 2-8℃, up to expiry date (12 months) |
| HRP Conjugate Diluent | 24T/48T/96T: 1 vial, 14 mL<br>96T*5: 5 vials, 14 mL | |
| Concentrated Wash Buffer(25×) | 24T/48T/96T: 1 vial, 30 mL<br>96T*5: 5 vials, 30 mL | |
| Substrate Reagent | 24T/48T/96T: 1 vial, 10 mL<br>96T*5: 5 vials, 10 mL | |
| Stop Solution | 24T/48T/96T: 1 vial, 10 mL<br>96T*5: 5 vials, 10 mL | |
| Plate Sealer | 24T/48T/96T: 5 pieces<br>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).

## 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

## 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 ± 10 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**

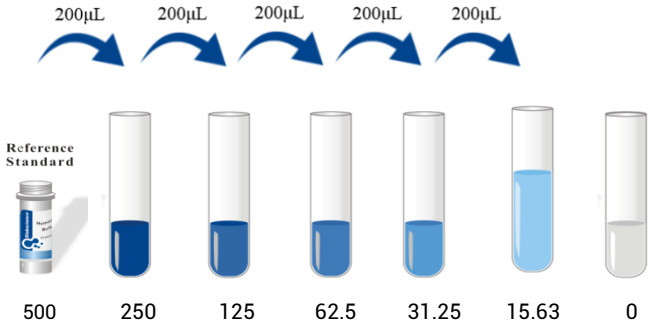
1) Tubes for blood collection should be disposable and be non-endotoxin.

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. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 0.4 mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 500 ng/mL(or add 0.4 mL of Reference Standard &Sample Diluent, let it stand for 1-2 min and then mix it thoroughly with a vortex meter of low speed. Bubbles generated during vortex could be removed by centrifuging at a relatively low speed). Then make serial dilutions as needed. The recommended dilution gradient is as follows: 500、250、125、62.5、31.25、15.63、0 ng/mL.
   Dilution method: Take 6 EP tubes, add 200 µL of Reference Standard & Sample Diluent to each tube. Pipette 200 µL of the 500 ng/mL working solution to the first tube and mix up to produce a 250 ng/mL working solution. Pipette 200 µL of the solution from the former tube into the latter one according to this step. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.




4. **HRP Conjugate working solution:** HRP Conjugate is HRP conjugated RBD. Calculate the required amount before the experiment (50 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP Conjugate 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.
5. **Samples**: Dilute the tested serum or plasma at 10 fold by using the Reference Standard & Sample Diluent, mix thoroughly.
6. **Positive control**: Dissolve Positive Control with 0.2 mL Reference Standard & Sample Diluent.
7. **Negative control**: Dissolve Negative Control with 0.5 mL Reference Standard & Sample Diluent.
8. Dissolved standard, pre-treated Samples and Controls should be stored at 2-8℃ and used within 1 day.

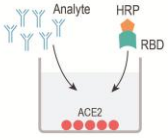
## Assay procedure

1. Determine wells for **diluted Standard**, **Blank**, **Positive/Negative Controls** and **Samples**. Add 50μL each dilution of standard, pre-treated Samples and Controls into the appropriate wells (It is recommended that all Samples, Standards and Controls be assayed in duplicate). Immediately add 50μL of

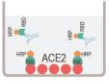
**HRP conjugated working solution** to each well. Cover the plate with the sealer provided in the kit. Incubate for 60 min at 37℃. Note: solutions should be added to the bottom of the micro TEST plate well, avoid touching the inside wall and causing foaming as much as possible.

2. Decant the solution from each well, add 350μL of **wash buffer** to each well. Soak for 30-60 seconds 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 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.
4. Add 50μL of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
5. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

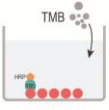
# Assay Procedure Summary



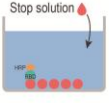

1. Add 50μL each dilution of Standard, pre-treated Samples and Controls. Immediately add 50μL of HRP-RBD working solution. Incubate for 60 min at 37℃.




2. Aspirate and wash the plate for 3 times.




3. Add 90μL of Substrate Reagent. Incubate for about 15 min at 37°C.




4. Add 50μL of Stop Solution.

5. Read the plate at 450nm immediately. Calculation of the results.

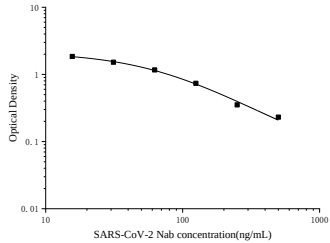
## Calculation of results

Average the duplicate readings for each standard and samples. Plot a four parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If the OD of the sample under the lowest limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

## Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.

| ng/mL | OD | Standard Curve |
|---|---|---|
| 500 | 0.231 |  |
| 250 | 0.352 | |
| 125 | 0.737 | |
| 62.5 | 1.165 | |
| 31.25 | 1.519 | |
| 15.63 | 1.853 | |
| 0 | 2.260 | |



## Quality control

For each assay, both Positive and Negative Controls must be included to validate the results. The value of each Control must meet the requirements as follows, otherwise, the test is invalid and should be repeated.

- Negative Control: ≤15.63 ng/mL.
- Positive Control: 50-200 ng/mL.

## Performance

### ■ Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level SARS -CoV-2 Nab were tested 20 times on one plate,

respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level SARS -CoV-2 Nab were tested on 3 different plates, 20 replicates in each plate, respectively.

| | Intra-assay Precision | | | Inter-assay Precision | | |
|---|---|---|---|---|---|---|
| Sample | 1 | 2 | 3 | 1 | 2 | 3 |
| n | 20 | 20 | 20 | 20 | 20 | 20 |
| Mean(ng/mL) | 17.7 | 110.98 | 413.33 | 18.21 | 117.00 | 424.56 |
| Standard deviation | 0.84 | 7.41 | 34.77 | 1.28 | 9.77 | 28.65 |
| CV (%) | 4.73 | 6.68 | 8.41 | 7.05 | 8.35 | 6.75 |

■ **Recovery**

The recovery of SARS-CoV-2 Nab spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

| Sample Type | Range (%) | Average Recovery (%) |
|---|---|---|
| Serum (n=8) | 90-101 | 94 |
| EDTA plasma (n=8) | 95-107 | 101 |
| Heparin Plasma (n=8) | 92-106 | 98 |

■ **Linearity**

Samples were spiked with high concentrations of SARS-CoV-2 Nab and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

| | | Serum (n=10) | EDTA plasma (n=10) | Heparin plasma (n=10) |
|---|---|---|---|---|
| 1:9 | Range (%) | 78-101 | 89-112 | 79-103 |
| | Average (%) | 91 | 101 | 92 |
| 1:18 | Range (%) | 82-97 | 80-101 | 82-115 |
| | Average (%) | 88 | 91 | 98 |
| 1:36 | Range (%) | 81-103 | 84-121 | 91-99 |
| | Average (%) | 91 | 103 | 95 |
| 1:72 | Range (%) | 79-95 | 91-108 | 86-107 |
| | Average (%) | 87 | 98 | 96 |

■ **Test data**

Samples (serum/plasma) from 121 randomly selected healthy volunteers (who had not been infected with SARS-CoV-2 and had not been vaccinated) and 77 vaccinators were verified with this kit:

Test data from 121 healthy volunteers (OD450)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1.871 | 1.867 | 1.971 | 1.855 | 2.142 | 2.234 | 2.236 | 2.257 | 2.018 | 2.259 | 2.057 |
| 2.225 | 1.904 | 2.062 | 1.836 | 1.84 | 2.204 | 1.856 | 2.04 | 2.274 | 1.913 | 2.184 |
| 2.263 | 2.014 | 1.983 | 1.97 | 1.878 | 2.237 | 2.086 | 1.948 | 2.151 | 1.925 | 2.218 |
| 2.114 | 2.146 | 2.27 | 2.041 | 1.859 | 1.953 | 1.859 | 2.131 | 1.853 | 1.855 | 2.092 |
| 1.893 | 2.215 | 2.199 | 2.252 | 1.934 | 2.052 | 1.834 | 1.825 | 1.897 | 2.206 | 2.088 |
| 1.918 | 2.038 | 2.044 | 2.008 | 2.23 | 1.992 | 2.208 | 1.851 | 2.161 | 2.005 | 1.895 |
| 1.905 | 2.211 | 2.225 | 2.012 | 1.965 | 2.238 | 2.202 | 1.885 | 2.084 | 2.223 | 1.901 |
| 1.886 | 2.255 | 2.151 | 1.955 | 2.001 | 2.172 | 2.193 | 2.108 | 1.823 | 1.938 | 2.009 |
| 2.003 | 1.842 | 1.923 | 2.013 | 2.038 | 1.921 | 1.907 | 1.98 | 2.028 | 1.934 | 2.069 |
| 2.25 | 2.204 | 2.234 | 2.085 | 2.104 | 1.828 | 1.854 | 2.083 | 2.07 | 1.863 | 2.084 |
| 2.246 | 1.838 | 2.047 | 1.898 | 2.044 | 2.251 | 2.099 | 2.05 | 2.124 | 1.838 | 1.975 |

Test data from 77 vaccinators (OD450):

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.815 | 0.573 | 1.175 | 1.234 | 1.552 | 0.682 | 1.148 | 0.647 | 0.944 | 0.443 | 0.945 |
| 1.419 | 0.333 | 0.505 | 0.91 | 1.097 | 0.929 | 0.422 | 1.136 | 0.959 | 1.002 | 0.873 |
| 1.196 | 1.553 | 0.335 | 1.442 | 0.924 | 1.554 | 0.112 | 0.811 | 1.337 | 1.045 | 1.433 |
| 0.907 | 0.999 | 0.773 | 0.531 | 0.65 | 1.195 | 1.532 | 0.943 | 1.015 | 0.261 | 0.113 |
| 0.846 | 0.382 | 0.937 | 1.507 | 1.051 | 1.452 | 0.492 | 0.936 | 0.145 | 0.692 | 0.631 |
| 1.047 | 0.397 | 0.45 | 0.497 | 1.544 | 0.464 | 0.662 | 0.618 | 1.069 | 0.985 | 1.318 |
| 0.497 | 0.115 | 0.307 | 0.318 | 0.951 | 0.349 | 0.377 | 0.627 | 0.194 | 0.333 | 0.185 |

## Troubleshooting

If the results are not good enough, please take pictures and save the experimental data in time. Keep the used plate and remaining reagents. Then contact our technical support to solve the problem. Meanwhile, you could also refer to the following table:

| Problem | Causes | Solutions |
|---|---|---|
| Poor standard curve | Inaccurate pipetting | Check pipettes. |
| | Improper standard dilution | Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing. |
| | Wells are not completely aspirated | Completely aspirate wells in between steps. |
| Low signal | Insufficient incubation time | Ensure sufficient incubation time. |
| | Incorrect assay temperature | Use recommended incubation temperature. Bring substrate to room temperature before use. |
| | Inadequate reagent volumes | Check pipettes and ensure correct preparation. |
| | Improper dilution | |
| | HRP conjugate inactive or TMB failure | Mix HRP conjugate and TMB, rapid coloring. |
| Deep color but low value | Plate reader setting is not optimal | Verify the wavelength and filter setting on the Microplate reader. |
| | | Open the Microplate Reader ahead to pre-heat. |
| Large CV | Inaccurate pipetting | Check pipettes. |
| High background | Concentration of target protein is too high | Use recommended dilution factor. |
| | Plate is insufficiently washed | Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed. |
| | Contaminated wash buffer | Prepare fresh wash buffer. |
| Low sensitivity | Improper storage of the ELISA kit | All the reagents should be stored according to the instructions. |
| | Stop solution is not added | Stop solution should be added to each well before measurement. |

## 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.