本试剂盒仅供体外研究使用，不用于临床诊断！

FineTest®

COVID-19 中和抗体（COVID-19 Neutralizing Antibody）ELISA 试剂盒

COVID-19 Neutralizing Antibody ELISA Kit

产品货号：EH4954

版本号: V4.0

包装规格：48T/96T

请勿将不同货号、不同批次号的试剂混用，否则试剂盒将无法正常工作

使用前请仔细阅读说明书。如果有任何问题，可通过以下方式联系我们：

销售部电话 027-86697005

技术部电话 18064071591（ELISA 售后）

技术部电话 18107218793（ELISA 售前）

电子邮箱 sales2@fn-test.com

网址 https://www.fn-test.cn/

复购时请提供产品批号(见试剂盒标签)，以便我们更高效地为您服务。

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。

如有更大包装需求可定制。

武汉菲恩生物科技有限公司.

湖北省武汉市东湖新技术开发区高新二路 388 号武汉光谷国际生物医药企业加速器 1.2 期 C22 栋一层、二层(430206)

技术支持相关文件

文件名称	样品制备指南	ELISA 实验操作流程	ELISA 实验手工洗板	TMB 显色精准控制	标曲和浓度计算软件 CurveExpert1.4(含使用教程)
网址	https://www.fn-test.cn/downloads/	http://www.fn-test.cn/videos/elisa-kit-video/	https://www.fn-test.cn/videos/elisa-experiment-how-to-hand-wash-plate-operation-video/	http://www.fn-test.cn/knowledge-share/tmb-color-rendering-precise-control/	https://www.fn-test.cn/knowledge-share/elisa-standard-curve-draw/
二维码					

性能介绍

用途	用于体外定量检测血清，血浆，细胞培养上清或其它生物样品中的 COVID-19 Neutralizing Antibody。		
适用种属	Universal	实验方法	双抗原夹心法
检测范围	1.563-100ng/ml	灵敏度	0.938ng/ml
检测时长	4 小时(不含平衡和样品准备时间)		
单孔样品最大用量	血清：50ul；血浆：50ul；细胞培养上清：100ul；细胞裂解液或组织裂解液: 100ul；其它液体样品：50ul		
特异性	特异性和 COVID-19 Neutralizing Antibody 结合，与其它类似物无明显交叉反应		
储存条件	未启封试剂盒 2-8°C(严禁冻存)，有效期见盒面标签		

背景简介

新冠病毒表面的刺突蛋白 S (spike，S)蛋白介导新冠病毒识别宿主细胞受体，促进膜融合，并诱导免疫反应产生中和抗体。新冠中和抗体的检测可以反映群体的感染率、个人的康复情况以及疫苗的效果。

检测原理

本试剂盒采用双抗原夹心 ELISA 法，实验时长 4 小时。试剂盒中的酶标板已预先包被抗原。将标准品、适度稀释的待测样品分别加入相应孔中,孵育后洗去未结合的成分。加入生物素-检测抗原，生物素-检测抗原与已经结合在包被抗原上的 COVID-19 Neutralizing Antibody 相结合。洗去未结合的成分后，加入 HRP-链霉亲和素(SABC)。再洗去未结合的成分，加入 TMB 显色底物，TMB 在辣根过氧化物酶(HRP)的催化下呈现蓝色，加反应终止液后变成黄色。用酶标仪在 450 nm 波长测 OD 值。通过绘制标准曲线计算样品中 COVID-19 Neutralizing Antibody 的浓度。目的物质的浓度与 OD450 值之间呈正比。

各组件及开启后保存条件

未启封的试剂盒，请保存在 2-8°C。开启后，保存条件见如下表格所示：

组件	名称	规格(48T)	规格(96T)	开启后保存条件
E001	Elisa 酶标板(可拆卸) ELISA Microplate(Dismountable)	8 孔×6 条	8 孔×12 条	将未使用的孔放入拉链铝箔袋中，并加入干燥剂，密封保存。可在 2-8°C 保存 1 个月；在-20°C 保存 6 个月。
E002	冻干标准品 Lyophilized Standard	1 支	2 支	将未使用的标准品放入干燥剂包中。可在 2-8°C 保存 1 个月；在-20°C 保存 6 个月。
E003	生物素-抗原（冻干品） Biotin-labeled Antigen	1 支	1 支	
E034	浓缩 HRP-链霉亲和素 100X HRP-Streptavidin Conjugate(SABC)	1 支 60ul	1 支 120ul	2-8°C (避光)
E024	TMB 显色底物 TMB Substrate	1 瓶 5ml	1 瓶 10ml	
E005	纯化水 Purified water	200ul	200ul	2-8°C
E039	样品稀释液 Sample Dilution Buffer	1 瓶 10ml	1 瓶 20ml	
E040	抗原稀释液 Antigen Dilution Buffer	1 瓶 5ml	1 瓶 10ml	
E049	SABC 稀释液 SABC Dilution Buffer	1 瓶 5ml	1 瓶 10ml	
E026	反应终止液 Stop Solution	1 瓶 5ml	1 瓶 10ml	
E038	浓缩洗涤液 25X Wash Buffer 25X	1 瓶 15ml	1 瓶 30ml	
E006	覆膜	3 张	5 张	
E007	说明书	1 份	1 份	

注意：试剂瓶内提供的液体试剂体积比标签标明的稍多。请使用移液器精确量取并做相应比例稀释。

所需器材和试剂

1、 酶标仪(波长 450nm 滤光片)

2、 37°C 恒温箱(不推荐使用细胞用 CO_2 培养箱)

3、 自动洗板机或多道移液器/5ml 滴管(手工洗板用)

4、 精密的单道(0.5-10μL, 5-50μL, 20-200μL, 200-1000μL)和多通道移液器(移液器使用前需校准)。

5、 无菌的 EP 管及一次性吸头

6、 吸水纸及加样槽

7、 去离子水或蒸馏水

样品采集及保存

以下样品处理步骤为精简操作，详细内容请查看第二页样品制备指南网址或二维码。

1、血清

全血样本室温放置 2 小时或 2-8°C 过夜。1000×g 离心 20 分钟，取上清。可立即检测，或按一次使用量分装冻存于-20°C 或-80°C。

2、血浆

抗凝剂推荐使用 EDTA-Na2/K2，样品采集后 30 分钟内于 2-8°C，1000×g 离心 15 分钟，取上清。可立即检测，或按一次使用量分装冻存于-20°C 或-80°C。其他抗凝剂的使用及选择请查看样品制备指南。

3、组织样本

组织样本一般制成组织匀浆，处理方法如下：

3.1、将目标组织置于冰上，用预冷的 PBS 缓冲液(0.01M，pH=7.4)洗涤去除残留的血液，称重后备用。

3.2、在冰上用裂解液研磨组织匀浆。加入裂解液的体积取决于组织的重量，一般情况每 1g 组织碎片使用 9ml 裂解液。另外建议在裂解液中加入蛋白酶抑制剂，如 1mM PMSF。

3.3、可再利用超声破碎或反复冻融进一步处理(超声破碎过程中，需冰浴降温；反复冻融法可重复 2 次)。

3.4、将制备好的匀浆液于 5000×g 离心 5 分钟，留取上清即可检测。或按一次使用量分装冻存于-20°C 或-80°C。

3.5、根据实验需要，组织匀浆样本可先测定总蛋白浓度,以便于数据分析，推荐 BCA 法。一般调整总蛋白浓度至 1-3mg/ml 用于 ELISA 检测。某些组织样本，如肝脏，肾脏，胰腺因含有较高浓度的内源性过氧物酶, 在样品浓度较高的情况下会和显色底物反应，出现假阳性。可尝试使用 1%H2O2 灭活 15min 再检测。

注意：裂解液常用 PBS 缓冲液，或使用中等强度 RIPA 裂解液。使用 RIPA 裂解液时，PH 值需调整为 PH7.3，避免使用含 NP-40，Triton X-100 和 DTT 的组分，会严重抑制试剂盒工作。推荐使用 50mM Tris+0.9%NaCL+0.1% SDS,PH 7.3，可自行配制或联系我们购买。

4、细胞培养上清

收集上清液，2-8°C，2500rpm 离心 5min，收集澄清的细胞培养上清。立即用于检测，或按一次使用量分装于-80°C 冻存备用。

5、细胞裂解液

5.1、悬浮细胞的收集及裂解：2-8°C，2500rpm 离心 5min，收集细胞。再加入预冷的 PBS 轻轻混匀清洗，2-8°C，2500rpm 离心 5min，收集细胞。加入 0.5-1ml 细胞裂解液及适量蛋白酶抑制剂(如 PMSF，工作浓度 1mmol/L)，置于冰上，裂解 30min-1h , 或者配合超声波破碎。

5.2、贴壁细胞的收集及裂解：吸走上清液，加入预冷的 PBS 洗三次。加入 0.5-1ml 细胞裂解液及适量蛋白酶抑制剂(如 PMSF，工作浓度 1mmol/L)，用细胞刮轻轻刮下贴壁细胞。细胞悬液转入离心管中，置于冰上，裂解 30min-1h，或者配合超声波破碎。

5.3、细胞裂解过程中可用枪头吹打或间断摇动离心管，使蛋白充分裂解, 出现黏糊状是 DNA，可以使用超声波破碎 DNA。(或用超声波 3-5mm 探头，功率 150-300W, 冰上超声处理样品，工作 1-2 秒，停止 30 秒， 3~5 个循环。)

5.4、裂解或超声破碎完成，2-8°C，10000rpm 离心 10min，上清移入 EP 管中，立即用于检测，或按一次使用量分装于 -80°C 冻存备用。

注意：注意事项同组织样本。

6、其他生物样本

2-8°C，1000×g 离心样品 20 分钟。收集上清液立即用于检测，或按 一次使用量分装于-80°C 冻存备用。

样本处理相关试剂推荐：100mM PMSF 蛋白酶抑制剂，货号 E051。FineTest Lysis Buffer(for ELISA)，货号：E050。

样品其它注意事项

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、 样品最佳保存条件：2-8°C 保存应小于 5 天，-20°C 不应超过 6 个月，-80°C 不应超过 2 年，超过以上时间应保存在液氮中。冻存的标本融化时，为了减少冰晶(0°C)对样品的破坏，应采用 15-25°C 水浴快速融化，融化后离心除去沉淀物，混匀后用于检测。

3、 试剂盒检测范围不等同于样本中待测物的浓度范围。如果样品中待测物浓度过高或过低，请对样本做适当的稀释或浓缩。

4、 若所检样本特殊，无参考数据，建议做预实验验证其有效性。

5、 重组蛋白可能与试剂盒中捕获或检测抗原不匹配而出现不能检测的情况。

试剂盒使用注意事项

1、 使用不同的试剂盒时，需先做好标记，防止组分混用，导致实验失败。

2、 试剂盒开启后，酶标板和标准品的保存条件请参考组件保存条件表格(受潮后活性会下降)。如发生使用或保存不当导致组件缺损，可申请购买配套组件(如 E002 冻干标准品)。

3、 请使用无菌一次性吸头吸取试剂，使用后，须旋紧试剂瓶盖，以防止微生物污染和蒸发。

4、 手工洗板时，加洗液的吸头或滴管，切勿接触酶标板孔。不充分的洗涤或污染容易造成假阳性和高背景。

5、 检测过程中，请提前准备好下一步实验所需试剂，洗板后及时将试剂加入板孔，防止板孔干燥，导致检测失效。

6、 在未经确认的情况下，请勿将其他批次试剂盒的试剂或其他来源的试剂用于本试剂盒。

7、 请勿重复使用一次性吸头，以免造成交叉污染。

8、 加样完成，贴覆膜以防孵育过程样品的蒸发，在推荐温度下完成孵育过程。

9、 试验中请穿实验服、戴口罩、手套等，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。

样品稀释方案推荐

以下表格为本试剂盒针对**有限样本**推荐的稀释比例，仅供参考。(ND 为未检出)

样本类型	推荐稀释比例	参考含量
Patients serum (1 month after recovery)	1/50-1/2000	0.3-16ug/ml
Healthy serum	1/2 dilution	ND

血清/血浆中的基质成分会影响检测结果，需用样品稀释液至少稀释 2 倍(1/2)！

如果您的模型组样本需要其他稀释比例，请参考如下通用稀释方案(此方案为检测不设置复孔的稀释方案。需要设置复孔时，请将样品及稀释液体积 x 复孔数)：

稀释 2 倍(1/2)：一步稀释。取 60ul 样品加入 60ul 样品稀释液中，轻轻混匀。

稀释 5 倍(1/5)：一步稀释。取 24ul 样品加入 96ul 样品稀释液中，轻轻混匀。

稀释 10 倍(1/10)：一步稀释。取 12ul 样品加入 108ul 样品稀释液中，轻轻混匀。

稀释 20 倍(1/20)：一步稀释。取 6ul 样品加入 114ul 样品稀释液中，轻轻混匀。

稀释 50 倍(1/50)：一步稀释。取 3ul 样品及 47ul 生理盐水(即 0.9%氯化钠)加入 100ul 样品稀释液中，轻轻混匀。

稀释 100 倍(1/100)：一步稀释。取 3ul 样品及 177ul 生理盐水加入 120ul 样品稀释液中，轻轻混匀。

稀释 1000 倍(1/1000)：两步稀释，可先稀释 50 倍(此步骤全使用生理盐水稀释)，再稀释 20 倍。轻轻混匀。

稀释 10000 倍(1/10000)：两步稀释，可先稀释 100 倍(此步骤全使用生理盐水稀释)，再稀释 100 倍。轻轻混匀。

稀释 100000 倍(1/100000)：三步稀释，可先稀释 50 倍，再稀释 20 倍(前两步全使用生理盐水稀释)，最后稀释 100 倍。轻轻混匀。

注意：每步稀释时取液量不少于 3ul，稀释倍数不超过 100 倍。每步稀释都需混合均匀，避免起泡。

检测前试剂准备

提前 20 分钟从冰箱中取出试剂盒，平衡到室温(18-25°C)。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及标准品，剩余酶标板条和标准品需按指定条件保存。

1、洗液:

用去离子水或蒸馏水(推荐电阻率为 18MΩ 的超纯水)将 30ml 浓缩洗涤液(48T 为 15ml)稀释至 750ml(48T 为 375ml)并混匀。或依实验所需，取适量浓缩洗涤液稀释至 25 倍体积并混匀，将未使用的溶液放回 2-8°C。

如果浓缩的洗涤液中形成了晶体，可以在 40°C 水浴中加热(加热温度不应超过 50°C)，直至晶体完全溶解，混匀后使用。

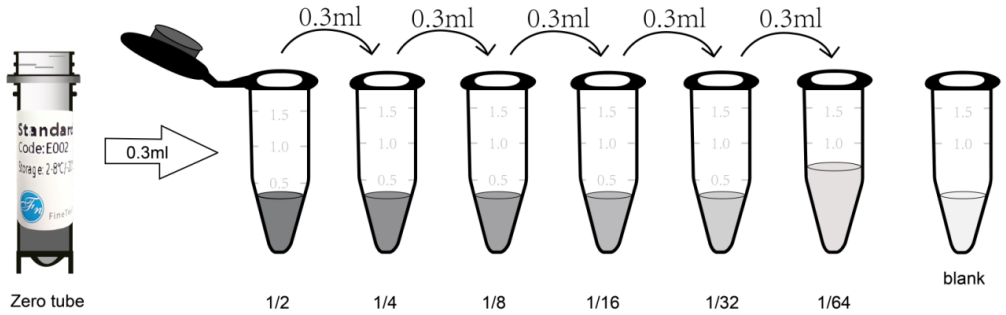
配制好的洗液，最好当天使用完，用不完的，可以保存在 2-8°C，不超过 48 小时。

2、标准品:

2.1、将冻干标准品管于 10000×g 离心 1 分钟。标记为 Zero tube.

2.2、取 1ml 样品稀释液加入冻干标准品管中，旋紧管盖，室温静置 2 分钟，上下颠倒数次轻轻混匀(或加入 1mL 样品稀释液，静置 1-2 分钟后，用低速涡旋仪混匀 3-5 秒)。1000×g 低速离心 1 分钟，将液体收集至管底。

2.3、梯度稀释：另取 7 个 EP 管，分别标记为 1/2、1/4、1/8、1/16、1/32、1/64 和 blank。先在每个 EP 管中分别加入 0.3ml 的样品稀释液。再取 0.3ml Zero tube 标准品溶液加入到 1/2 管中，充分混合。再取 0.3ml 1/2 管标准品溶液到 1/4 管中，充分混合。再取 0.3ml 1/4 管标准品溶液到 1/8 管中，充分混合，依此类推。注意 Blank EP 管中仅有样品稀释液。此时，从 Zero tube 管到 blank 管这 8 个 EP 管中标准品的浓度分别为 100ng/ml, 50ng/ml, 25ng/ml, 12.5ng/ml, 6.25ng/ml, 3.125ng/ml, 1.562ng/ml, 0ng/ml。



Prepare standard solutions

注: 已溶解的零号管标准品，请保存于 2-8°C，并在 12 小时内使用。已稀释过的其他梯度标准品工作液请于 2 小时内使用。

3、生物素-抗原工作液：

工作液实验前 30 分钟内准备好，现用现配，不适合长期存放。

3.1、复溶：2000×g 离心 1 分钟，将浓缩生物素-抗原收集至管底。取纯化水 130ul，加入生物素标记抗原管中，完全溶解并混匀后，1000×g 低速离心 1 分钟，置于 2-8°C 保存。

3.2、计算所需工作液的总体积：100ul/孔×孔数。(最好准备比总体积多 100ul-200ul 的量)

3.3、用抗原稀释液按 1/100 的比例稀释浓缩生物素-抗原，充分混匀。(如将 10ul 浓缩生物素-抗原加入 990ul 抗原稀释液中)

4、HRP-链霉亲和素(SABC)工作液：

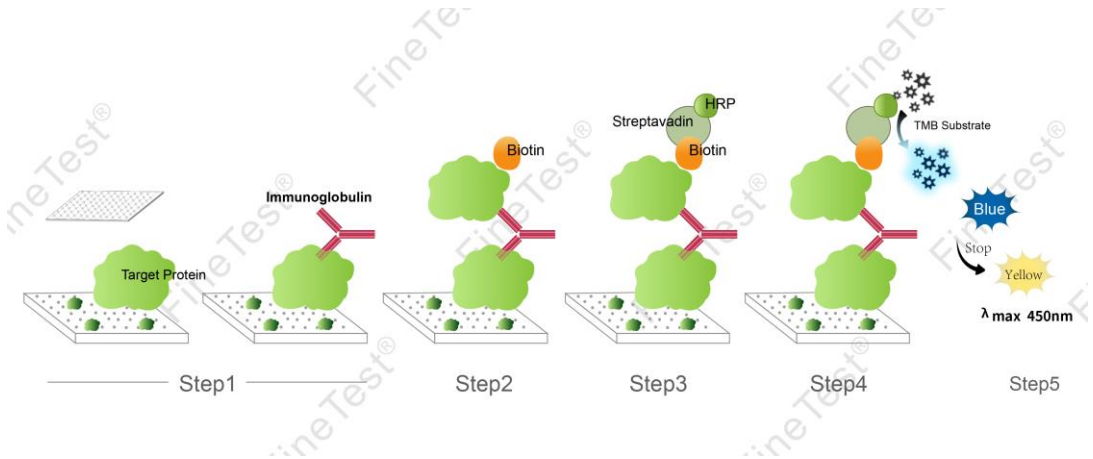
实验前 30 分钟内准备好，现用现配，不适合长期存放。

4.1、计算所需工作液的总体积：100ul/孔×孔数。(最好准备比总体积多 100ul-200ul 的量。)

4.2、1000×g 低速离心 1 分钟，将浓缩 SABC 收集至管底。

4.3、用 SABC 稀释液按 1/100 的比例稀释浓缩 SABC，充分混匀。(如将 10ul 的浓缩 SABC 加入 990ul SABC 稀释液中)

操作步骤概要



步骤 1：向孔中加入 100ul 标准品或待测样品，贴上覆膜， 37°C 静置孵育 90 分钟。

洗板：洗板 2 次。不浸泡。

步骤 2：加入 100ul 生物素-抗原工作液，贴上覆膜， 37°C 静置孵育 60 分钟。

洗板： 洗板 3 次。每次浸泡 1 分钟。

步骤 3：加入 100ul HRP-链霉亲和素(SABC)工作液，贴上覆膜， 37°C 静置孵育 30 分钟。

洗板：洗板 5 次。每次浸泡 1 分钟。

步骤 4： 添加 90ul TMB 显色底物。贴上覆膜， 37°C 静置孵育 10-20 分钟(请使用 TMB 显色精准控制方法)。

步骤 5：添加 50ul 反应终止液。立即在 450nm 处读取 OD450 值并计算。

详细操作步骤

稀释样品和试剂时，需将它们完全混合。建议每次测试都绘制标准曲线。

1、 设定标准品孔、样品孔、空白孔，并记录其位置。为减小实验误差，建议将标准品和样品设置复孔。

2、 **加样：**向标准品孔中加入 100ul 各梯度标准品，向样品孔中加入 100ul 适度稀释的待测样品，向空白孔中加入 100ul 样品稀释液。贴上覆膜，并在 37°C 下静置孵育 90 分钟。(将溶液添加到微孔板的底部，轻轻晃 动混匀，并尽可能避免接触管壁和起泡。)

3、 **洗板 2 次：**取下覆膜，吸去或甩掉酶标板内的液体，在洁净的吸水纸上拍 2-3 次。每孔加入洗涤缓冲液 350ul，不浸泡，弃掉孔内液体，在吸水纸上拍 2-3 次。重复此洗板步骤 2 次。

4、 **加生物素-抗原工作液：**向每孔加入 100ul 生物素-抗原工作液。贴上覆膜，37°C 静置孵育 60 分钟。

5、 **洗板 3 次:** 取下覆膜，吸去或甩掉酶标板内的液体，在洁净的吸水纸上拍 2-3 次。每孔加入洗涤缓冲液 350ul，浸泡 1 分钟，弃掉孔内液体，在吸水纸上拍 2-3 次。重复洗板步骤 3 次。

6、 **加 HRP-链霉亲和素(SABC)：**向每孔加入 100ul SABC 工作液。贴上覆膜，37°C 静置孵育 30 分钟。(同时将整瓶 TMB 放入 37°C 温箱中平衡)

7、 **洗板 5 次：**取下覆膜，用洗涤缓冲液洗板 5 次，方法参考步骤 5。

8、 **加 TMB 显色底物：**向每孔加入 90ul TMB 显色底物，贴上覆膜，在 37°C 避光静置孵育 10-20 分钟。打开酶标仪预热 15min。(注意：不可使用配制 HRP 偶联物的加样槽。显色根据颜色的实际变化，反应时间可以缩短或延长，但不能超过 30 分钟。当标准孔中出现较好的蓝色梯度时，可以终止反应。显色强度不易太弱或过强，精准控制显色方法请查阅说明书第二页相关文件及二维码)

9、 **加反应终止液：**显色后，孔内液体不可弃掉，向每孔加入 50ul 反应终止液。颜色将由蓝色立即变为黄色。添加终止液的顺序与添加 TMB 底物的顺序相同。

10、 **OD 值的测量：**立即用酶标仪在 450nm 处读取 OD450 数值。（如果您的酶标仪有可以选择的校正波长，则设置为 570nm 或 630nm。校正读数值为 OD450 的值减去 OD570 或 OD630 的值。这种方式可以校正并去除非显色物质的 OD 值，从而获得更准确的检测结果。如果酶标仪没有 570nm 或 630nm 波长，则可使用原始 OD450 值。）

结果计算

（操作视频：https://www.fn-test.cn/videos/standard-curve-drawing-video-in-elisa-kit/）

1、 取标准品和样品复孔的平均 OD450 值（使用原始 OD450 值或校正读数值），再减去空白孔的 OD450 值，作为计算值。

2、 以浓度为横坐标，OD450 值为纵坐标，可使用四参数方程 4PL 绘制标准曲线(作图时去掉空白孔的值)。也可使用酶标仪自带的作图软件(如 Thermo FC 型号酶标仪 SkanIt RE 软件)，或 Curve Expert 1.3 or 1.4 专业软件(本公司网站可以免费下载使用)绘制标准曲线。

3、 将样本的 OD450 值代入标准曲线，即可计算得到样品的浓度值。 如果样品被稀释过，则需乘以相应的稀释倍数。

不同方法绘制标准曲线的说明

1、 线性图：一个坐标轴表示抗原的浓度，另一个表示读数 OD450 值。R^2 值在此通常用于确定拟合，数值大于 0.99 表示拟合非常好。然而，线性图往往会压缩曲线下端上的数据点，导致计算结果不准确。

2、 半对数图：帮助抵消线性图引起的下端压缩。半对数图使用浓度的对数与读数的关系。这种方法通常会得到数据点分布更均匀的 s 形曲线。

3、 对数/双对数图：为低到中浓度范围提供良好的线性。但范围的高端则容易失去线性。

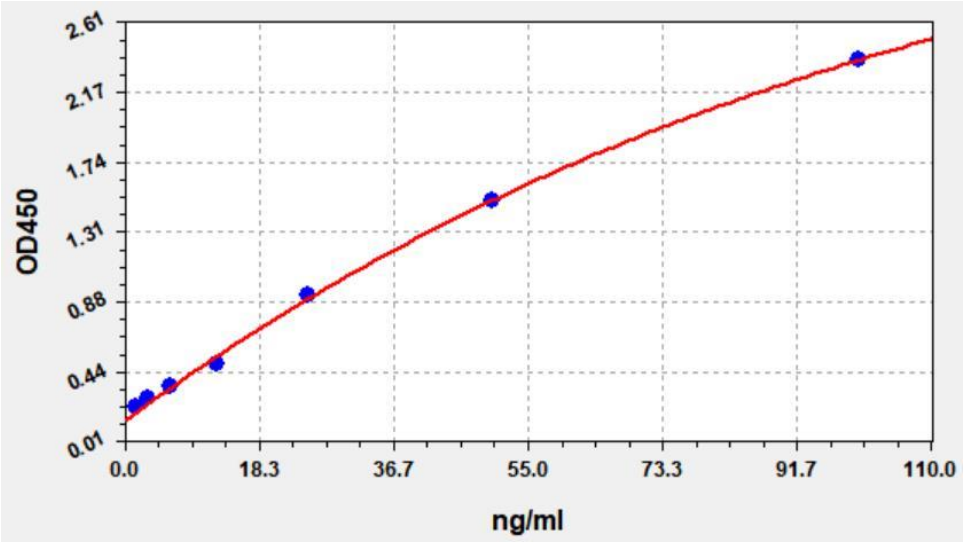
4、 四或五参数方程(4PL 或 5PL)曲线：方法更复杂，考虑了其他参数，比如最大值和最小值，因此需要更复杂的计算。4PL 假设拐点周围对称，而 5PL 考虑了不对称的情况，通常更适合免疫分析。如果您的软件允许，则 4-PL 和 5-PL 将适用于大部分 ELISA 校正标准曲线。

实验数据及标准曲线

本产品经品管部检测，符合使用手册的性能要求。(实验室湿度为 20%-60%，温度为 18°C -25°C。显色前将 TMB 平衡至 37°C，加入酶标板孔后，37°C 避光孵育 15 分钟。)

因具体实验环境及操作存在差异，以下实验数据和标准曲线仅供参考，实验人员需根据自己的实验建立标准曲线。

STD.(ng/ml)	OD-1	OD-2	Average	Corrected
0	0.086	0.087	0.087	0
1.562	0.231	0.233	0.232	0.146
3.125	0.285	0.272	0.279	0.192
6.25	0.365	0.348	0.357	0.27
12.5	0.512	0.484	0.498	0.412
25	0.952	0.889	0.921	0.834
50	1.581	1.422	1.502	1.415
100	2.331	2.426	2.379	2.292



精密度

板内精密度：低、中、高浓度样本分别在同 1 块酶标板上检测 20 次。

板间精密度：低、中、高浓度样本分别在 3 块酶标板上检测 20 次。

类别	板内变异系数			板间变异系数		
样本	1	2	3	1	2	3
数量	20	20	20	20	20	20
平均值(ng/ml)	3	12.48	48.46	3.23	12.9	49.77
标准差	0.15	0.79	2.84	0.16	0.54	2.92
变异系数(%)	4.98	6.32	5.86	4.87	4.2	5.86

回收率

将一定含量的 COVID-19 Neutralizing Antibody 加入样本中，并通过将测量值与样品中 COVID-19 Neutralizing Antibody 的预期量进行比较来计算回收率。

样品类型	回收率范围 (%)	平均回收率 (%)
血清(n=10)	90-102	95
EDTA 血浆(n=10)	85-100	95
肝素血浆(n=10)	86-104	91

线性

将添加有适当浓度 COVID-19 Neutralizing Antibody 的样品分别稀释 2 倍、4 倍、8 倍来检测，得出回收率范围。

样品类型	1:2	1:4	1:8
血清((n=10)	82-100%	81-100%	90-98%
EDTA 血浆(n=10)	82-98%	84-95%	81-100%
肝素血浆(n=10)	85-100%	82-98%	88-99%

稳定性

未拆封的试剂盒在 37°C 和 2-8°C 进行稳定性实验，得出稳定性数据。

试剂盒(n=5)	37°C 一个月	2-8°C 六个月
平均值 (%)	80	95-100

ELISA 疑难解答提示

若实验结果不理想，请及时将显色结果拍照并保存实验数据，保留所用板条及未使用试剂。凭试剂盒盒面货号及批次号，联系我公司销售人员为您解决问题。同时您也可以参考以下表格自查原因：

问题描述	可能原因	相应对策
标准曲线无信号	检测试剂加样顺序不对	确认各步骤所加试剂正确，可重做一次并确认
	混淆了不同试剂盒的组件	使用试剂盒本身的各组件，可重做一次并确认
	漏加试剂	确认试剂是否添加
标准曲线显色过强	混淆了不同试剂盒的组件，或配置工作液浓度过高	使用试剂盒本身的各组件，可重做一次并确认
标准曲线图形不好	曲线选择不恰当	尝试使用不同方法绘制曲线
样品无信号	待测样品含量低于测定的检测限	减小稀释倍数或浓缩样品
	目的物和缓冲液的相容性不好	确保样品储存缓冲液与待测样品相容性
	样品制备不正确	参考样品制备指南并规范保存
	样品保存时间过久或反复冻融	按一次使用量分装并规范保存
变异系数 (CV) 较大	显色时在孔中形成沉淀	增大样品的稀释倍数
	酶标板不干净	实验时勿碰触酶标板底部
	孔中有气泡	确保酶标板读数时孔中无气泡
	板孔洗涤不均	检查洗板机的管口是否畅通
	试剂未混匀	所有试剂已充分混合
	移液量不一致	使用校准好的移液器和正确的移液方法
标准曲线信号弱	标准品复溶不当	开盖前短暂离心冻干标准品管；检查是否溶解完全
	标准品已降解	按推荐方式保存标准品
	移液体积出错或不准	使用校准好的移液器和正确的移液方法
	试剂盒过期	不使用过期产品
	试剂盒保存不当	按说明书要求保存各组分
	板孔过分干燥	检测及加样过程不可中断。尤其洗板之后，需及时加入试剂。孵育时，贴覆膜。
	酶反应显色慢	每次使用前将整瓶 TMB 显色底物在 37°C 平衡 30min。延长孵育时间
	酶标仪波长不正确	核实波长并重新读取 OD450 值
	板孔清洗过度	按说明书描述的洗板次数
背景高	未彻底洗涤	按说明书描述的洗板次数

	洗涤缓冲液受污染	洗涤液现用现配，手工洗板悬空加洗液，不触及板孔
	检测试剂过多，或配置浓度过高	使用校准好的移液器和正确的移液方法
	终止后读数不及时	加入终止液后立即读数
	TMB 底物孵育在强光下进行	显色时避光孵育

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。

(For Research Use Only. Not For Use In Diagnostic Procedures!)

FineTest®

COVID-19 Neutralizing Antibody ELISA Kit

Catalogue No.: EH4954

Revision: V4.0

Size: 48T/96T

Please do not mix and use reagents from different kits or different batches. Otherwise, it might not work properly.

Please read the manual carefully before use. Feel free to contact us if you have any questions.

Tel 027-86697005

Email sales2@fn-test.com

Website https://www.fn-test.cn/

Please provide the batch number (see kit label) for more rapid response and services.

It's strongly recommended to use this kit within the expiry date printed on the kit label.

Wuhan Fine Biotech Co., Ltd

1-2 Floor, BLD 22, Optics Valley Biopharmaceutical Accelerator,

No.388 Gaoxin 2nd Road, East Lake High Tech Development Zone,

Wuhan, 430074, Hubei, China (430075)

Fax: (0086)027-87800889

Technical support related documents

Title of Document	Sample preparation guide	Experimental operation procedure	TMB color rendering control	Standard curve and concentration calculation software CurveExpert1.4(Including tutorial)
Website	https://www.fn-test.com/content/uploads/2022/06/ELISA-Sample-Preparation-Protocol-2022.6.6.pdf	https://www.fn-test.com/videos/elisa-test/	https://www.fn-test.com/videos/targeted-control-of-tmb-coloring/	https://www.fn-test.com/content/uploads/2019/08/CurveExpert-1.4.zip
Quick Mark				

Product Features

Application	In vitro quantitative determination of COVID-19 Neutralizing Antibody concentrations in serum, plasma, cell culture supernatant and other biological samples.		
Reactivity	Universal	Detection Method	Sandwich
Range	1.563-100ng/ml	Sensitivity	0.938ng/ml
Detection Duration	4 hours(excluding balancing and sample preparation)		
Samples needed for single well(Max)	Serum: 50 ul, Plasma: 50 ul, Cell Culture Supernatant: 100ul, cell or tissue lysate: 100ul, Other liquid samples: 50ul		
Specificity	Specifically recognize COVID-19 Neutralizing Antibody, no obvious cross reaction with other analogues		
Storage	2-8°C (for sealed box), please do not freeze! See kit label for expiry date		

Background

The S (spike, S) protein on the surface of the novel coronavirus mediates the novel coronavirus recognition of host cell receptors, promotes membrane fusion, and induces an immune response to produce neutralizing antibodies. Testing for neutralizing antibodies to COVID-19 can reflect the infection rate of the population, the recovery of the individual and the effectiveness of the vaccine.

Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Antigen was pre-coated onto the 96-well plate. The biotin conjugated antigen was used as the detection antigen. The standards and pilot samples were added to the wells subsequently. After incubation, unbound conjugates were removed by wash buffer. Then, biotinylated detection antigen was added to bind with COVID-19 Neutralizing Antibody conjugated on coated antigen. After washing off unbound conjugates, HRP-Streptavidin was added. After a third washing, TMB substrates were added to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that turned yellow after adding acidic stop solution. Read the O.D. absorbance at 450nm in a microplate reader. The concentration of target antigen in the sample is positively correlated with OD450 and can be calculated by plotting the standard curve.

Kit Components and Storage

The sealed kit can be stored at 2-8 ℃. The storage condition for opened kit is specified in the table below:

No.	Item	Size(48T)	Size(96T)	Storage Condition for Opened Kit
E001	ELISA Microplate(Dismountable)	8×6	8×12	Put the rest strips into a sealed foil bag with the desiccant. Stored for 1 month at 2-8°C; Stored for 6 month at -20°C
E002	Lyophilized Standard	1vial	2vial	Put the rest standards into a desiccant bag. Stored for 1 month at 2-8°C; Stored for 6 month at -20°C
E003	Biotin-labeled Antigen (Lyophilized)	1vial	1vial	
E034	HRP-Streptavidin Conjugate(SABC, 100X)	60ul	120ul	2-8°C (Avoid Direct Light)
E024	TMB Substrate	5ml	10ml	
E005	Purified water	200ul	200ul	2-8°C
E039	Sample Dilution Buffer	10ml	20ml	
E040	Antigen Dilution Buffer	5ml	10ml	
E049	SABC Dilution Buffer	5ml	10ml	
E026	Stop Solution	5ml	10ml	
E038	Wash Buffer(25X)	15ml	30ml	
E006	Plate Sealer	3 pieces	5 pieces	
E007	Product Description	1 copy	1 copy	

Note: The liquid reagent bottle contains slightly more reagent than indicated on the label. Please use pipette accurately measure and do proportional dilution.

Required Instruments and Reagents

1. Microplate reader (wavelength: 450nm)
2. 37°C incubator (CO_2 incubator for cell culture is not recommended.)
3. Automated plate washer or multi-channel pipette/5ml pipettor (for manual washing purpose)
4. Precision single (0.5-10µL, 5-50µL, 20-200µL, 200-1000µL) and multi-channel pipette with disposable tips(calibration is required before use.)
5. Sterile tubes and Eppendorf tubes with disposable tips
6. Absorbent paper and loading slot
7. Deionized or distilled water

Sample Collection and Storage

The following sample processing steps are concise operations. For detailed sample preparation guideline, please refer to the Quick Mark or the link (https://www.fn-test.com/content/uploads/2022/06/ELISA-Sample-Preparation-Protocol-2022.6.6.pdf).

1. Serum

Place whole blood sample at room temperature for 2 hours or at 2-8°C overnight. Centrifuge for 20min at 1000xg and collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

2. Plasma

EDTA-Na_2/K_2 is recommended as the anticoagulant. Centrifuge samples for 15 minutes at 1000×g 2-8°C within 30 minutes after collection. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay. For other anticoagulant types and uses, please refer to the sample preparation guideline.

3. Tissue Sample

Generally tissue samples are required to be made into homogenization. Protocol is as below:

3.1. Place the target tissue on the ice. Remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Then weigh for usage.

3.2. Use lysate to grind tissue homogenates on the ice. The adding volume of lysate depends on the weight of the tissue. Usually, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS (e.g. 1mM PMSF).

3.3. Do further process using ultrasonic disruption or freeze-thaw cycles (Ice bath for cooling is required during ultrasonic disruption; Freeze-thaw cycles can be repeated twice.) to get the homogenates.

3.4. Homogenates are then centrifuged for 5 minutes at 5000×g. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

3.5. Determine total protein concentration by BCA kit for further data analysis. Usually, total protein concentration for Elisa assay should be within 1-3mg/ml. Some tissue samples such as liver, kidney, pancreas which containing a higher endogenous peroxidase concentration may react with TMB substrate causing false positivity. In that case, try to use 1% H_2O_2 for 15min inactivation and perform the assay again.

Notes: PBS buffer or the mild RIPA lysis can be used as lysates. While using RIPA lysis, make the PH=7.3. Avoid using any reagents containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT due to their severe inhibition for kits' working. We recommend using 50mM Tris+0.9%NaCL+0.1%SDS, PH7.3. You can prepare by yourself or contact us for purchasing.

4. Cell Culture Supernatant

Collect the supernatant: Centrifuge at 2500 rpm at 2-8℃ for 5 minutes, then collect clarified cell culture supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

5. Cell Lysate

5.1. Suspension Cell Lysate: Centrifuge at 2500 rpm at 2-8℃ for 5 minutes and collect cells. Then add pre-cooling PBS into collected cell and mix gently. Recollect cell by repeating centrifugation. Add 0.5-1ml cell lysate and appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Lyse the cell on ice for 30min-1h or disrupt the cell by ultrasonic disruption.

5.2. Adherent Cell Lysate: Absorb supernatant and add pre-cooling PBS to wash three times. Add 0.5-1ml cell lysate and appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Scrape the adherent cell with cell scraper. Lyse the cell suspension added in the centrifuge tube on ice for 30min-1h or disrupt the cell by ultrasonic disruption.

5.3. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Mucilaginous product is DNA which can be disrupted by ultrasonic cell disruptor on ice. (3~5mm probe, 150-300W, 3~5 s/time, 30s intervals for 1~2s working).

5.4. At the end of lysate or ultrasonic disruption, centrifuge at 10000rpm at 2-8℃ for 10 minutes. Then, the supernatant is added into EP tube to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

Notes: Read notes in tissue sample.

6. Other Biological Sample

Centrifuge samples for 15 minutes at 1000×g at 2-8℃. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

Recommended reagents for sample preparation: Cat No: E051 100mM PMSF protease inhibitor, Cat No: E050 FineTest Lysis Buffer (for ELISA).

Notes for Samples

1. Blood collection tubes should be disposable and non-endotoxin. Avoid to use hemolyzed and lipemia samples.

2. The best sample storage condition: less than 5 days at 2-8℃; within 6 months at -20℃; within 2 years at -80℃. Stored in liquid nitrogen for a longer storage. When melting frozen samples, rapid water bath at 15-25℃ can decrease the effect of ice crystal (0℃) on the sample. After melting, centrifuge to remove the precipitate, and then mix well.

3. The detection range of this kit is not equivalent to the concentration of analyze in the sample. For analyses with higher or lower concentration, please properly dilute or concentrate the sample.

4. Pretest is recommended for special samples without reference data to validate the validity.

5. Recombinant protein may not match with the capture or detection antigen in the kit, resulting in the undetectable assay.

Precautions for Kits

1. When using different Elisa kits, labeling is required to avoid mixed components and failed assay.

2. After opening the kit, please refer to the table of storage condition for coated plate and standards (Dampness may decrease the activity.). If any component is missing or damaged during the assay or storage, please contact us for ordering a new one to replace.(e.g. E002 lyophilized standard)

3. Sterile and disposable tips are required during the assay. After use, the reagents bottle cap has to be tightened to avoid the microbial contamination and evaporation.

4. While manual washing, please keep tips or pipettors for adding wash buffer away from the well. Insufficient washing or contamination easily causes false positive and high background.

5. During the assay, prepare required reagents for next step in advance. After washing, add the reagent into the well in time to avoid dryness. Otherwise, dry plate will result in the failed assay.

6. Before confirmation, reagents from other batches or sources should not be used in this kit.

7. Don't reuse tips and tubes to avoid cross contamination.

8. After loading, seal the plate to avoid the evaporation of the sample during incubation. Complete the incubation process at recommended temperature.

9. Please wear the lab coat, mask and gloves to protect yourself during the assay. Especially, for the detection of blood or other body fluid samples, please follow regulations on safety protection of biological laboratory.

Recommended Sample Dilution Ratio

Please refer to the following table of recommended dilution ratio for limited samples for reference. (ND: Not Detected)

Sample Type	Recommended Dilution Ratio	Content
Patients serum (1 month after recovery)	1/50-1/2000	0.3-16ug/ml
Healthy serum	1/2 dilution	ND

The matrix components in serum/plasma will affect the test results, which it need to be diluted at least 1/2 with Sample Dilution Buffer before testing!

If other dilution ratio for your sample model is required, please refer to the universal dilution ratio below. (The ratio is suitable for single-well assay. For duplicate assay, please follow the calculation: volume of sample and diluent x number of duplicate well)

For 2 fold dilution (1/2): One step dilution. Add 60ul sample into 60ul sample diluent and mix gently.

For 5 fold dilution (1/5): One step dilution. Add 24ul sample into 96ul sample diluent and mix gently.

For 10 fold dilution (1/10): One step dilution. Add 12ul sample into 108ul sample diluent and mix gently.

For 20 fold dilution (1/20): One step dilution. Add 6ul sample into 114ul sample diluent and mix gently.

For 50 fold dilution (1/50): One step dilution. Add 3ul sample and 47ul normal saline (0.9% NaCl) into 100ul sample diluent and mix gently.

For 100 fold dilution (1/100): One step dilution. Add 3ul sample and 177ul normal saline into 120ul sample diluent and mix gently.

For 1000 fold dilution (1/1000): Two step dilution. Create a 50-fold dilution first (normal saline is used throughout the dilution). Then, create a 20-fold dilution and mix gently.

For 10000 fold dilution (1/10000): Two step dilution. Create a 100-fold dilution first (normal saline is used throughout the dilution). Then, create the same dilution again and mix gently.

For 100000 fold dilution (1/100000): Three step dilution. Create a 50-fold dilution and 20-fold dilution respectively (normal saline is used in the first two steps.) Finally, create a 100-fold dilution and mix gently.

Notes: The volume in each dilution is not less than 3ul. Dilution factor should be within 100 fold. Mixing during dilution is required to avoid foaming.

Reagent Preparation and Storage

Take the Elisa kit from the fridge around 20 minutes earlier and equilibrate to room temperature(18-25℃). For repeated assays, please just take the strips and standards required for the current assay, store the rest materials according to the relevant condition.

1. Wash Buffer

Dilute 30ml (15ml for 48T) concentrated wash buffer to 750ml (375ml for 48T) wash buffer with deionized or distilled water and mix well. (The recommended resistivity of ultrapure water is 18MΩ.) Alternatively, take appropriate amount of concentrated wash buffer according to the assay requirement, then create a 25-fold dilution and mix well. Store the rest solution at 2-8℃.

Crystals formed in the concentrated wash buffer can be heated by water bath at 40℃ till complete dissolution. (Heating temperature should be below 50℃.) Mix well for the next step. It's better to use up the prepared wash buffer in one day. Store the rest buffer at 2-8℃ within 48h.

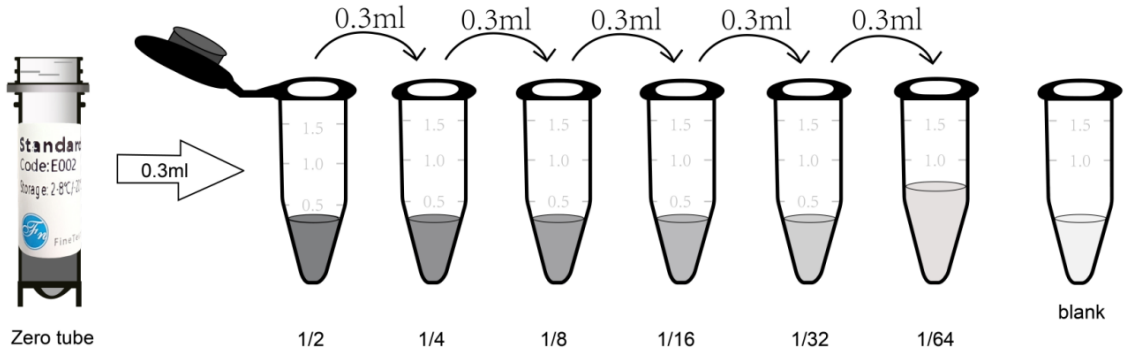
2. Standards

2.1. Centrifuge standards tube for 1min at 10000xg. Label it as Zero tube.

2.2. Add 1ml sample dilution buffer into the standard tube. Tighten the tube cap and Let it stand for 2min at room temperature. Invert the tube several times to mix gently. (Or you can mix it using a low speed vortex mixer for 3-5 seconds.)

2.3. Centrifuge the tubes for 1min at 1000xg, making the liquid towards the bottom of tube and removing possible bubbles.

2.4. Standard dilution: Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the sample dilution buffer into each tube. Add 0.3ml solution from zero tube into 1/2 tube and mix them thoroughly. Transfer 0.3ml from 1/2 tube into 1/4 tube and mix them thoroughly. Transfer 0.3ml from 1/4 tube into 1/8 tube and mix them thoroughly, so on till 1/64 tube. Now blank tube only contain 0.3ml sample dilution buffer. The standard concentration from zero tube to blank tube is 100ng/ml, 50ng/ml, 25ng/ml, 12.5ng/ml, 6.25ng/ml, 3.125ng/ml, 1.562ng/ml, 0ng/ml.



Prepare standard solutions

Notes: Store the zero tube with dissolved standards at 2-8℃ and use it within 12h. Other diluted working solutions containing standards should be used in 2h.

3. Preparation of Biotin-labeled Antigen Working Solution

The working solution should be prepared within 30min before the assay and can't be stored for a long time.

3.1. **Dissolve**: Centrifuge for 1min at 2000xg and bring down the concentrated biotin-labeled antigen to the bottom of tube. Add 130ul purified water into tube and mix them thoroughly, after the biotin-labeled antigen is dissolved, please store it at 2-8°C.

3.2. Calculate required total volume of the working solution: 100ul/well x quantity of wells. (It's better to prepare additional 100ul-200ul.)

3.3. Dilute the biotinylated detection antigen with antigen dilution buffer at 1/100 and mix them thoroughly. (e.g. Add 10ul concentrated biotin-labeled antigen into 990ul antigen dilution buffer.)

4. Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution

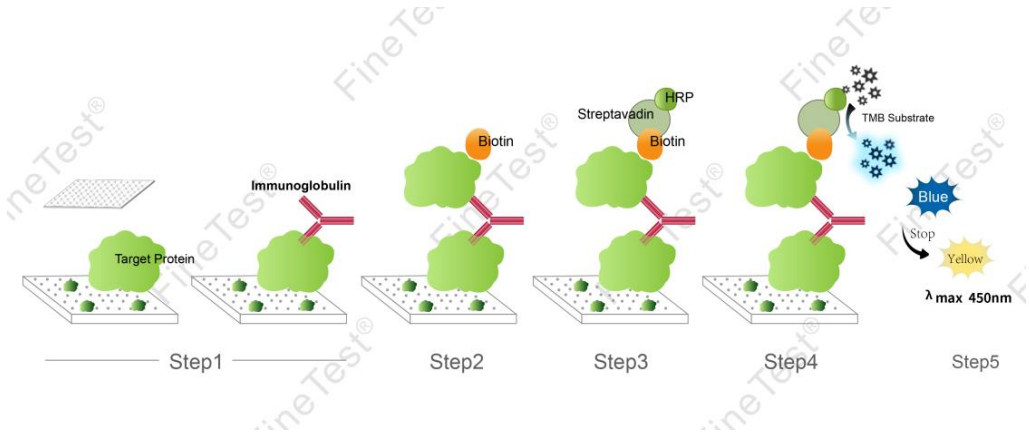
The working solution should be prepared within 30min before the assay and can't be stored for a long time.

4.1. Calculate required total volume of the working solution: 100ul/well x quantity of wells. (It's better to prepare additional 100ul-200ul.)

4.2. Centrifuge for 1min at 1000xg in low speed and bring down the concentrated SABC to the bottom of tube.

4.3. Dilute the concentrated SABC with SABC dilution buffer at 1:99 and mix them thoroughly. (e.g. Add 10ul concentrated SABC into 990ul SABC dilution buffer.)

Assay Procedure Summary



Step 1: Add 100ul standard or sample into each well, seal the plate and static incubate for 90 minutes at 37°C.

Washing: Wash the plate twice without immersion.

Step 2: Add 100ul biotin-labeled antigen working solution into each well, seal the plate and static incubate for 60 minutes at 37°C.

Washing: Wash the plate three times and immerse for 1min each time.

Step 3: Add 100ul SABC working solution into each well, seal the plate and static incubate for 30 minutes at 37°C.

Washing: Wash the plate five times and immerse for 1min each time.

Step 4: Add 90ul TMB substrate solution, seal the plate and static incubate for 10-20 minutes at 37°C. (Accurate TMB visualization control is required.)

Step 5: Add 50ul stop solution. Read at 450nm immediately and calculate.

Detailed Assay Procedure

When diluting samples and reagents, they must be mixed completely. It's recommended to plot a standard curve for each test.

1. Set standard, pilot samples, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It's recommended to measure each standard and sample in duplicate to decrease experimental errors.

2. Standards and samples loading: Aliquot 100ul of zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube, 5th tube, 6th tube into each standard well. Also add 100ul sample dilution buffer into the control (blank) well. Then, add 100ul pilot samples into each sample well. Seal the plate and static incubate for 90 minutes at 37°C. (Add the solution to the bottom of each well. Mix gently and without touch the sidewall and foam the sample.)

3. Wash twice: Remove the cover, then absorb the liquid in the plate or tap the plate on a clean absorbent paper two or three times. Add 350ul wash buffer into each well without immersion. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step twice.

4. Biotin-labeled Antigen: Add 100ul biotin-labeled antigen working solution into each well. Seal the plate and static incubate for 60 minutes at 37°C.

5. Wash three times: Remove the cover, then absorb the liquid in the plate or tap the plate on a clean absorbent paper two or three times. Add 350ul wash buffer into each well and immerse for 1min. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step three times.

6. HRP-Streptavidin Conjugate (SABC): Add 100ul SABC working solution into each well. Seal the plate and static incubate for 30 minutes at 37°C. (Put the whole bottle of TMB into the 37°C incubator to equilibrate for 30min.)

7. Wash five times: Remove the cover, and then wash the plate with wash buffer five times. Read washing method in step 5.

8. TMB Substrate: Add 90ul TMB Substrate into each well, seal the plate and static incubate at 37°C in dark within 10-20 minutes. Run the microplate reader and preheat for 15min.

(Notes: Please do not use the reagent reservoirs used by HRP couplings. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells. Weaker or stronger color intensity is unacceptable. Please refer to TMB color rendering control in page 2 or QR code for detail.)

9. Stop: Keep the liquid in the well after staining. Add 50ul stop solution into each well. The color will turn yellow immediately. The order for adding stop solution and TMB substrate solution is the same.

10. OD Measurement: Read the O.D. absorbance at 450nm in a microplate reader immediately. (If your microplate reader has a choice of correction wavelength, set it to 570nm or 630nm. Correct the read value to the OD450 value minus the OD570 or OD630 value. In this way, the OD value of non-chromogenic substances can be corrected and removed, thus obtaining more accurate results. If the microplate reader does not have a 570nm or 630nm wavelength, the original OD450 value can be used.)

Calculation of Results

（Operate Video：https://www.fn-test.com/videos/elisa-sample-concentration-calculation/）

1. Calculate the mean OD450 value (using the original OD450 value or the corrected OD450 value) of the duplicate readings for each standard, control, and sample. Then, obtain the value of calculation by subtracting the OD450 blank.

2. Create a four parameter logistic curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. (Remove the OD450 blank during plotting.) Alternatively, you can use the curve fitting software offered by the microplate reader (e.g. Thermo SkanIt RE software, Curve Expert 1.3 or 1.4 available in FineTest website).

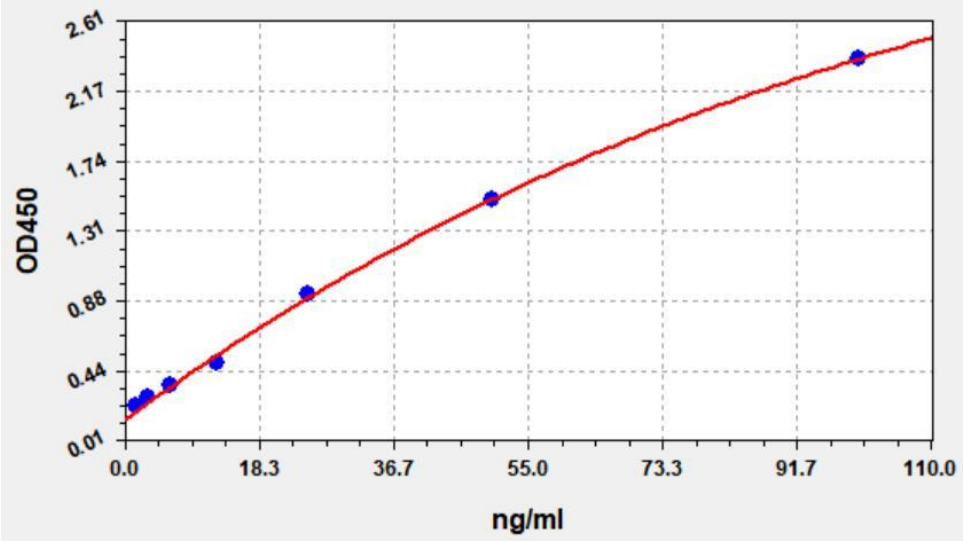
3. Calculate the sample concentration by substituting OD450 value into the standard curve. Diluted samples should be multiplied by the relevant dilution ratio.

Typical Data & Standard Curve

This product has been tested by Quality Control Department and meets performance specifications mentioned in the manual. (The humidity in the laboratory is 20%-60%, and the temperature is 18°C - 25°C. TMB was balanced to 37°C before color development, and incubated at 37°C for 15 minutes in the dark after adding the enzyme label plate holes.)

The following assay data are provided for reference, since experimental environment and operation are different. The establishment of standard curve depends on your own assay.

STD.(ng/ml)	OD-1	OD-2	Average	Corrected
0	0.086	0.087	0.087	0
1.562	0.231	0.233	0.232	0.146
3.125	0.285	0.272	0.279	0.192
6.25	0.365	0.348	0.357	0.27
12.5	0.512	0.484	0.498	0.412
25	0.952	0.889	0.921	0.834
50	1.581	1.422	1.502	1.415
100	2.331	2.426	2.379	2.292



Precision

Intra-assay Precision: samples with low, medium and high concentration are tested 20 times on same plate.

Inter-assay Precision: samples with low, medium and high concentration are tested 20 times on three different plates.

Item	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/ml)	3	12.48	48.46	3.23	12.9	49.77
Standard deviation	0.15	0.79	2.84	0.16	0.54	2.92
CV(%)	4.98	6.32	5.86	4.87	4.2	5.86

Recovery

Add a certain amount of COVID-19 Neutralizing Antibody into the sample. Calculate the recovery by comparing the measured value with the expected amount of COVID-19 Neutralizing Antibody in the sample.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	90-102	95
EDTA Plasma(n=5)	85-100	95
Heparin Plasma(n=5)	86-104	91

Linearity

Dilute the sample with a certain amount of COVID-19 Neutralizing Antibody at 1:2, 1:4 and 1:8 to get the recovery range.

Sample	1:2	1:4	1:8
Serum(n=5)	82-100%	81-100%	90-98%
EDTA Plasma(n=5)	82-98%	84-95%	81-100%
Heparin Plasma(n=5)	85-100%	82-98%	88-99%

Stability

Perform the stability test for the sealed kit at 37°C and 2-8°C and get relevant data.

Elisa kit(n=5)	37°C for 1 month	2-8°C for 6 months
Average (%)	80	95-100

ELISA Troubleshooting

If the ELISA result is unsatisfied, please take a screenshot for the staining result and store the OD data. Keep used strips as well the rest reagents. Contact us to solve your problem with the kit's catalogue number and batch number. You can also refer to the following table to check the reason.

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect order for adding reagents	Confirm the required reagent added in each step. Also repeat the assay and verify.
	Use components from different kits	Use the component included in the same kit. Also repeat the assay and verify.
	Forget to add some reagents	Verify whether the required reagent is added.
Overflow OD	Use components from different kits, or prepare the working solution with higher concentration	Use the component included in the same kit. Also repeat the assay and verify.
Poor standard curve	Inappropriate curve fitting model	Try to plot the curve by different fitting models.
Samples without signal	The amount of pilot sample is lower than the detection range.	Decrease dilution ratio or concentrate the sample.
	The detection target is incompatible with the buffer.	Verify the compatibility of sample storage buffer with the pilot sample.
	Incorrect preparation of sample	Please refer to sample preparation guideline and regularly store.
	Longer storage of sample or freeze-thaw cycle	Aliquot and store samples according to the assay requirement.
High CV%	Precipitate is formed in the well during staining.	Increase the dilution ratio of the sample.
	Unclean plate	Don't touch the bottom of the plate during the assay.
	Foam is found in the well.	Avoid foaming during reading in a microplate reader.
	Each well is washed unevenly.	Check whether the tube of the washer is smooth.
	Reagents are not completely mixed.	Mix all reagents completely.
	Inconsistent pipetting	Use calibrated pipette and correct pipetting method.
Standard curve with low signal	Standards are improperly reconstituted.	Before opening, shortly centrifuge the lyophilized standard tube till complete dissolution.
	Standards have been degraded.	Follow suggested storage conditions for

		standards.
	When pipetting, the required volume is incorrect or inaccurate.	Use calibrated pipette and correct pipetting method.
	Expired kit	Don't use expired products.
	Improper storage	Follow suggested storage conditions for all components.
	The well is over dried.	The assay and sample loading process can't be terminated. Especially after washing the plate, add reagents immediately. Seal the plate during incubation.
	Slow colorimetric reaction	Before use, equilibrate the whole bottle of TMB substrate for 30min at 37°C. Extend the incubation time.
	The wavelength of the microplate reader is incorrect.	Check the wavelength and read the OD450 value again.
	The well is washed excessively.	Follow suggested washing times in this manual.
High Background	Insufficient washing	Follow suggested washing times in this manual.
	Wash buffer is contaminated.	Use the prepared wash buffer immediately. During manual washing, add wash buffer without touching the well.
	Too many detection reagents or higher concentration.	Use calibrated pipette and correct pipetting method.
	Reading of assay result is not in time.	Read the assay result immediately after adding the stop solution.
	TMB substrate is incubated in strong light.	During colorimetry, incubate in the dark.

Declaration

1. Limited to current conditions and scientific techniques, all raw materials are not completely identified and analyzed. This product may have a technology-related quality risk.

2. During the Elisa kit development, some endogenous interferons(not all) in the biological sample have been removed or decreased.

3. The final assay result is related to the validity of reagents, experimental operation and environment. FineTest is only responsible for this kit, excluding sample consumption during using this kit. Before use, please consider and prepare enough samples required by the assay.

4. To get a satisfied assay result, please use all reagents offered by this kit. Don't use any product from other vendors. Strictly follow instructions of this manual.

5. During assay procedure, incorrect reagents preparation and parameter setting of the microplate reader may result in the abnormal result. Before assay, please read this manual carefully and adjust instruments.

6. Even if the assay is performed by the same person, results in two independent assays may be different. Thus, each step in the assay should be controlled to ensure the reproducibility.

7. Before delivery, this kit is subject to the strict QC. Influenced by transportation conditions and experimental devices, the assay result got by the customer may be different from original data. Inter-assay CV between different batches may be caused by reasons before.

8. This kit is not compared to similar kits from other vendors or methods for testing the same detection target. Thus, assay results may be inconsistent.

9. This kit allows for research use only. For IVD or other purposes, FineTest is not responsible for relevant consequences and doesn't bear any legal liability.