Human CENPC ELISA Kit

<table>
<tr><td>种　　属</td><td colspan="3">人</td><td colspan="2">检测范围</td><td colspan="3">0.156→10ng/ml</td></tr>
<tr><td>规　　格</td><td colspan="3">96T/48T</td><td colspan="2">灵 敏 度</td><td colspan="3">0.052ng/ml</td></tr>
<tr><td>有 效 期</td><td colspan="3">6 个月</td><td colspan="2">保存温度</td><td colspan="3">4℃</td></tr>
<tr><td>重 复 性</td><td colspan="3">板内板间变异系数<10%</td><td colspan="2">批　　号</td><td colspan="3">详见试剂盒外包装</td></tr>
<tr><td>特 异 性</td><td colspan="8">可检测样本中人的 CENPC，且与其类似物无明显交叉反应。</td></tr>
<tr><td rowspan="2">标准曲线
（ng/ml）</td><td>S1</td><td>S2</td><td>S3</td><td>S4</td><td>S5</td><td>S6</td><td>S7</td><td>Black</td></tr>
<tr><td>10.0</td><td>5.0</td><td>2.5</td><td>1.25</td><td>0.625</td><td>0.312</td><td>0.156</td><td>0</td></tr>
<tr><td>用　　途</td><td colspan="8">本试剂盒用于体外定性或定量分析，仅用于科研，不能用于临床诊断，适用于血清、血浆、组织匀浆、细胞培养上清及其它生物体液。</td></tr>
</table>

简介：着丝粒蛋白 C1 是着丝粒自身抗原，是内动粒板的一个组成部分。这种蛋白质是维持适当的动粒大小和及时过渡到后期所必需的。

使用前请仔细阅读说明书并检查试剂盒成分，有什么问题请及时与我们联系。

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一、检测原理：

上海威奥生物生产的 ELISA 试剂盒采用“夹心法”:将捕获抗体包被于酶标板上，捕获样品及标准品中的靶蛋白，生物素化的检测抗体与靶蛋白结合，SABC 复合物与生物素化检测抗体结合，形成免疫复合物，加入 TMB 显色液后，若反应孔中有靶蛋白则显蓝色，加入终止液变黄色，检测过程中未结合的成分均被洗去，用酶标仪在 450 nm 处测 OD 值，靶蛋白浓度与 OD 值之间呈正比，通过绘制标准曲线计算出标本中靶蛋白的浓度，从而对检测样品进行定性或相对定量分析。

二、试剂盒组分：（保存温度 4℃）

名 称	规格（48T）	规格（96T）
预包被酶标板	8×6 条	8×12 条
标准品 S（10×）	1 支	1 支
标准品/样品稀释液	15ml	15ml
生物素化检测抗体（100×）	1 支	1 支
生物素化检测抗体稀释液	6ml	12ml
SABC 复合物（100×）	1 支	1 支
SABC 复合物稀释液	6ml	12ml
TMB 显色液 A	3ml	6ml
TMB 显色液 B	3ml	6ml
终止液	3ml	6ml
30×浓缩洗涤液	30ml	30ml
封板胶纸	2 张	4 张
产品说明书	1 份	1 份

※需要而未提供的试剂和器材

1. 标准规格酶标仪、自动洗板机、恒温箱 。

2. 进口品牌可调节移液器及吸头，0.5-10μL，2-20μL，20-200μL，200-1000μL。一次检测样品较多时，最好用多通道移液器。
3. 干净的试管和 Ep 管。
4. 双蒸水或去离子水。

三、样本收集方法：

●**血清：** 室温血液自然凝固 10-20 分钟后，离心 20 分钟左右（2000-3000 转/ 分），收集上清-20℃或者-80℃ 冷冻保存。

●**血浆：** 应根据试剂盒的要求选择 EDTA、柠檬酸钠或肝素作为抗凝剂，抗凝血离心 20 分钟左右（2000-3000 转/分），仔细收集上清，-20℃或者-80℃冷冻保存。

●尿液、胸腹水、脑脊液、肺泡灌洗液：用无菌管收集，离心 20 分钟左右（2000-3000 转/分），仔细收集上清，-20℃或者-80℃冷冻保存。

●**细胞培养上清：** 用于检测分泌性的成分，用无菌管收集，离心 20 分钟左右（2000-3000 转/分），仔细收集上清，检测细胞内的成分时，用 PBS 稀释细胞悬液，细胞浓度达到 100 万/ml 左右，通过超声破碎或反复冻融(利用液氮和 37℃水浴对样品进行两到三次快速的冻融)，以使细胞破坏并放出细胞内成份，离心 20 分钟左右（2000-3000 转/分），仔细收集上清（如需要可取部分进行 BCA 蛋白定量），-20℃或 者-80℃ 冷冻保存。

●**组织标本：** 切割标本后，准确称取组织重量。按重量(g):体积(mL)=1:9 的比例，加入 9 倍体积的匀浆介质 PBS，用手工或匀浆器将标本匀浆充分，离心 20 分钟左右（2000-3000 转/分），仔细收集匀浆上清（如需要可取部分进行 BCA 蛋白定量），-20℃或者-80℃冷冻保存。

四、样本收集注意事项：

1. 每个标本量收集体积＝约 60μl×检测指标，如要做复孔，标本量收集体积＝约 60μl× 检测指标×2 ，更多复孔以此类推。
2. 收集标本前必须清楚要检测的成份是否足够稳定，以确定样本保存温度，保存条件参考样本保存。

3. 血清标本采集时，应注意避免溶血，红细胞溶解时会释放出具有过氧化物酶活性的物质， 以 HRP 为标记的 ELISA 测定中，溶血标本可能会增加非特异性显色。
4. 为了保证尿液检测结果的准确性，必须正确收集尿液标本和保存，盛尿容器要清洁干燥。最好使用一次性的容器（如塑料尿杯），避免因用药并清洗不干净而造成的污染，影响检测结果，尿液标本必须新鲜，留取后，应及时检测或保存，以免细菌繁殖，因在室温（尤其是夏季）中久置后尿中的磷酸盐等可析出结晶而干扰检测。
5. 冻结标本融解后，蛋白质局部浓缩，分布不均，应充分轻缓混匀，避免气泡，可上下颠倒混和，不要在混匀器上强烈振荡。
6. 混浊或有沉淀的标本应先离心或过滤，澄清后再检测。
7. 反复冻融会使蛋白效价降低，所以待测标本如需保存作多次检测，宜少量分装冰存。也可加入适当防腐剂。
8. 激素类标本需添加抑肽酶。

五、样本保存：

1. 4℃保存：1-4 天检测的样本，超过时间的需低温保存。
2. -20℃或-80℃保存：对收集后当天进行检测的标本，储存在 4℃备用，如有特殊原因需要周期收集标本， 将标本及时分装后放在 -20℃或-80℃条件下保存。避免反复冻融。
3. 一般情况下，标本 4℃可保存 48 小时，-20℃下可保存 1 个月，-80℃下可保存 6 个月。

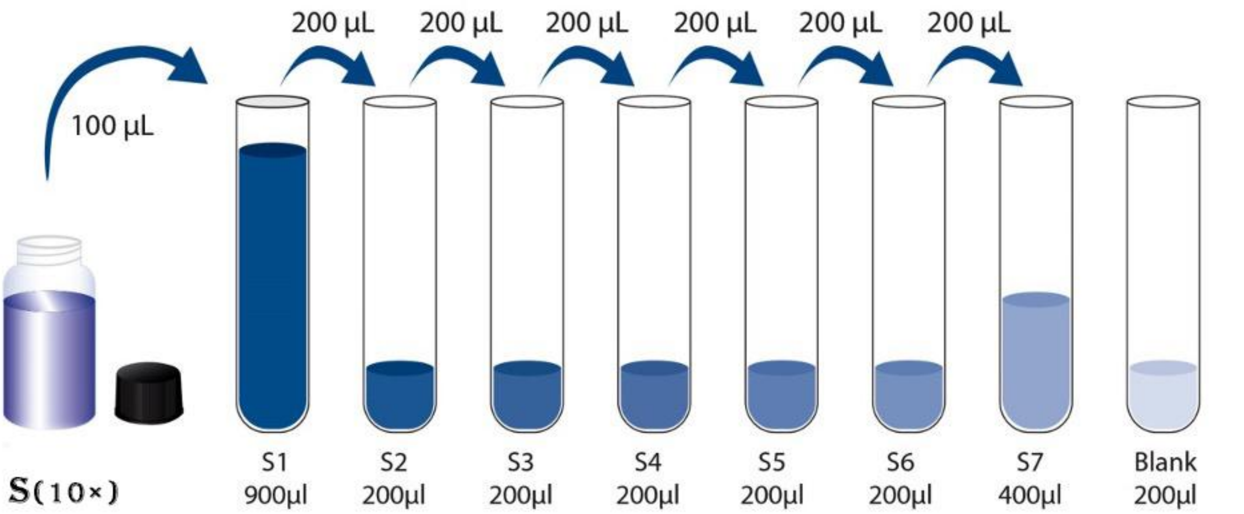
六、实验操作注意事项：

1. 在试验中标准品和样本检测时建议作双孔检测，每次检测都应做标准曲线。
2. 洗涤过程很关键，洗涤不充分将导致精确度误差及 OD 值错误地升高，从冰箱中取出的浓缩洗涤液可能 有结晶,属于正常现象，37℃水浴使结晶完全溶解后再配制洗涤液。
3. 检测时所有试剂都要恢复到室温，板条开封后剩余板条需封好，放回袋中 1 个月内用完。

4. 试剂盒使用超敏 TMB 溶液，显色过深时会出现沉淀状，属正常现象，混匀即可，不影响结果判读。
5. 试验中请穿着实验服并戴乳胶手套做好防护工作。
6. 不同批号的试剂盒组份不能混用(反应终止液除外)。
7. 试验中所用的 EP 管和吸头均为一次性使用，严禁混用。

七、检测前试剂准备:

1. 提前 20 分钟从冰箱中取出试剂盒，平衡至室温，读数前 15 分钟打开酶标仪预热。
2. 洗涤液配置：用蒸馏水 1:30 稀释（例：1ml 浓缩洗涤液加入 29ml 的蒸馏水）。
3. 标准品配制：取 8 个 1.5ml 离心管，分别标注 S1,S2,S3,S4,S5,S6，S7,Blank,第一管 S1 中加入标准品/样品稀释液 900μl，S2 至 Blank 中分别加入标准品/样品稀释液 200μl，在第一管 S1 中加入标准品 S（10×）溶液 100μl 置于漩涡混合器上混匀后用加样器吸出 200μl，移至 S2，如此反复作对倍稀释至 S7,混匀，Blank 为空白对照。（标准品的用量也可根据自己需要配置）



4. 生物素化抗体工作液配置:使用前 20 分钟，用生物素化抗体稀释液将 100×生物素化抗体稀释成 1×工作液，根据所需用量配置，当日使用，剩余弃之。
5. SABC 复合物工作液配置:使用前 20 分钟，用 SABC 复合物稀释液将 100×浓缩 SABC 复合物稀释成 1×工作液，当日使用，剩余弃之。
7. TMB 显色液的配置：使用前 10 分钟，将 TMB 显色液 A 液和 B 液 1：1 混合，避光放置备用。
8. 如果您检测的样本中靶蛋白浓度高于标准品最高值，建议重新检测，请根据实际情况，适当倍数稀释(建议做预实验，以确定稀释倍数)。

9. 当标准品/样品稀释液及洗涤液不够用时，可以用 1*PBST 替代。

八、洗板方法：

● **手工洗板方法：**

吸去（不可触及板壁）或甩掉酶标板内的液体；在实验台上铺垫几层吸水纸，酶标板朝下用力拍几次；将 1×洗涤缓冲液至少 300μl 注入孔内，浸泡 1-2 分钟。根据需要，重复此过程数次。

●**自动洗板：**

全自动洗板机的使用应注意以下几点：

1. 洗板前，应检查洗液瓶、蒸馏水瓶是否充足，废液瓶是否满瓶。
2. 在自检过程中注意观察洗液灌注是否通畅，排液是否通畅。
3. 在洗板过程中，应注意观察反应孔每孔是否灌满且无外溢，每孔吸水是否吸尽，并 且要保证洗液在孔中放置的时间。

九、检测程序:

1. **加样**：空白孔加入 50μl 标准品/样品稀释液，其余孔各对应加入标准品或待测样品 50μl，贴上封板胶纸，将反应板混匀后置 37℃，50 分钟。
2. **洗板**：用 1×洗涤液将反应板充分洗涤 3 次，每孔加入 1×洗液 300μl，每次震荡/浸泡 1-2 分钟，向滤纸上印干。
3. **加抗体**：空白孔加入 100μl 生物素化抗体稀释液，其余孔各加入 1×的生物素化抗体工作液 100μl，贴上封板胶纸，混匀后置 37℃，50 分钟。
4. **洗板**：同上。
5. **加 SABC**：每孔加入 SABC 复合物工作液 100μl，贴上封板胶纸，混匀后置 37℃，30 分钟。
6. **洗板**：同上。
7. **加显色液**：每孔加入提前配置好的 TMB 混合液 100μl，混匀后贴上封板胶纸，置于 37℃暗处反应 10-20 分钟（具体显色时间根据显色结果而定）。
8. **加终止液**：每孔加入 50μl 终止液，混匀，30 分钟内用酶标仪在 450nm 处测吸光值。

十、检测程序总结:

1. 加样品及标准品，37℃反应 50 分钟。洗涤 3 次。
2. 加生物素化检测抗体，37℃反应 50 分钟。洗涤 3 次。
3. 加 SABC 复合物，37℃反应 30 分钟。洗涤 3 次。
4. 加 TMB 显色液，37℃反应 10-20 分钟。
5. 加入终止液，读数。

十一、结果判断与计算：

1. 所有 OD 值建议减除空白孔值后再进行计算，如空白孔 OD 低于 0.1，也可以直接计算。
2. 以标准品浓度作横坐标，OD 值作纵坐标，手工绘制或用软件绘制标准曲线，根据样品 OD 值计算出相应含量，再乘以稀释倍数即可。

十二、声明：

1、本公司只对试剂盒本身负责，不对因使用该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的可能使用量，预留充足的样本。

2、若所检样本不包含在说明书所列样本之中，建议进行预实验验证其有效性，并注意留存样本。

3、使用化学裂解液制备的组织匀浆或细胞提取液可能会由于某些化学物质的引入导致 ELISA 实验结果偏差。

4、若样本为细胞培养上清，因该类样本干扰因素较多，如：细胞状态、细胞数量、采样时间等，所以可能存在检测不出的情况。

5、某些天然蛋白或重组蛋白，包括原核及真核重组蛋白，可能因为与本产品所使用的检测抗体及捕获抗体不匹配，而不被检测出。

6、建议使用新鲜样本，保存时间过长可能会存在蛋白降解或变性导致实验结果偏 差。

局限于现有条件及科学水平，尚不能对所有的原料进行全面的鉴定和分析，本产品可能 存在一定的质量技术风险，最终实验结果及试剂的有效性和操作者、温度等有密切相关，请务必准备充足的待测样品备用。

当说明书中英文不一致时，请依中文说明书为准。

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：

标准曲线较差

原因	解决方案
标准品溶液配置有误	确认是否进行正确稀释。
标准品复溶不当	开盖前进行离心;检查复溶后是否存在不溶物。
标准品已降解	按推荐方式保存和处理标准品。
曲线的标度不适合	尝试使用不同标度绘制曲线。
移液器加样误差	正确使用经过校准的移液。

无信号

原因	解决方案
孵育时间过短	样品在 4 ℃孵育过夜，或遵循试剂的实验方案。
靶标含量低于检测范围	减小样品的稀释倍数或浓缩样品。
样品类型不适用	对于没有验证过的样品类型，检测信号可能减弱或没有使用验证过的样品类型作为阳性对照同时进行检测。
抗原表位被孔板吸附，无法识别	使用直接或间接 ELISA 方法增强检测肽的能力，将肽偶联到大的载体蛋白上，然后包被到微量滴定板。
检测缓冲液的相容性	确保检测缓冲液与靶标兼容(例如，保留酶活性、保留蛋白质相互作用)。
检测试剂不足	遵循试剂的实验方案，增加检测试剂的浓度或用量。
样品制备不正确	确保进行正确的样品制备/稀释。样品可能与微量滴定板测定形式不兼容。
抗体不足	尝试不同的抗体浓度/稀释。
孵育温度过低	确保在正确温度下进行孵育。所有试剂(包括孔板)在进行实验前应处于室温，或试剂的实验方案所建议的温度。
波长不正确	确认波长，再次读板。
孔板被强力洗涤	检查并确保自动洗涤系统的压力正确。如果手动洗涤，则轻轻吸取冲洗缓冲液。
孔变干	测定开始后，不要让孔变干。将所有的孵育步骤使用封口膜或胶带密封孔板。
酶反应的显色速度慢	使用前配制底物溶液。确保母液未过期、未污染。延长孵育时间。
试剂盒没有充分平衡	试剂室温平衡至少 20 分钟，确保所有试剂已平衡至室温。

变异系数（CV）较大

原因	解决方案
孔中有气泡	读板前，确保不存在气泡。
孔洗涤不均/未充分洗涤	检查洗板机的所有管口是否畅通。使用推荐方法进行洗涤。
试剂混匀不充分	确保所有试剂充分混匀。
移液量不一致	正确使用经过校准的移液器
边缘效应	确保孔板和所有试剂处于室温。
样品制备或保存条件不一致	确保样品制备保持一致，使用最优的样品保存条件(例如尽可能减少反复冻融)。

背景偏高

原因	解决方案
孔洗涤不充分	按照实验方案建议进行洗涤。
洗涤缓冲液污染	制备新鲜的洗涤缓冲液。
检测试剂过多	确保试剂被正确稀释或者减少检测试剂的推荐浓度。
封闭缓冲液无效(例如检测试剂结合封闭剂;孔未完全封闭)	尝试不同的封闭剂和/或将封闭剂添加到洗涤缓冲液。
孵育/洗涤缓冲液的盐浓度	增加盐浓度可能会降低非特异性和/或减弱脱靶相互作用。
读板前加入终止液后时间太长	添加终止液后立即读板。
抗体出现非特异性结合	使用适当的封闭缓冲液，例如 BSA 或 5-10% 正常血清，如果是直标一抗，使用与一抗种属相同的血清，如果是非直标一抗，则使用与二抗种属相同的血清。确保孔已经过预处理，以防止非特异性附着。
高抗体浓度	尝试不同的稀释度，以获得最优结果。
底物孵育在光下进行	底物孵育应避光进行，或根据试剂的实验方案建议进行。
底物加入后孔中有沉淀生成	增大样品的稀释倍数或降低底物浓度。
孔板脏	清洁孔板底部。
显色液变质或者试剂过期	检查试剂盒有效期,在有效期内使用
孵育时间和温度的改变	按照说明书上推荐的时间和温度操作
盖板、容器或者枪头的重复使用	及时更换使用过的盖板，容器或者枪头

灵敏度偏低

原因	解决方案
ELISA 试剂盒保存不当	按推荐方式保存所有试剂。请注意，各试剂的保存条件可能有所不同。
靶标不足	浓缩样品或降低样品稀释度。
检测试剂失活	确保报告酶/荧光素具有预期的活性。
酶标仪设置不正确	在检测中，确保酶标仪设置为正确的吸收波长或激发/发射波长。
测定方法不够灵敏	更换更灵敏的检测系统(例如从比色检测转变为化学发光/荧光检测)。更换更灵敏的测定方法(例如从直接 ELISA 方法转变为夹心 ELISA 方法)。延长孵育时间或升高温度。
微量滴定板吸附靶标的效果不佳	将靶标共价结合到微量滴定板。
底物不足	加入更多底物。
样品类型不兼容(例如血清与细胞提取物)	对于没有验证过的样品种属，检测信号可能减弱或没有。使用验证过的样品类型作为阳性对照同时进行检测。
缓冲液或样品成分干扰	确认试剂中是否存在干扰性化合物。例如，抗体中的叠氮化钠会抑制 HRP 酶，在血浆中用作抗凝剂的 EDTA 会抑制酶反应。
混合或混用不同试剂盒的试剂	避免混合来自不同试剂盒的试剂。
试剂盒没有充分平衡	试剂室温平衡至少 20 分钟，确保所有试剂已平衡至室温。

Human CENPC Elisa Kit

Reactivity	Human	**Range**	0.156→10ng/ml
Size	96T/48T	**Sensitivity**	0.052ng/ml
Validity	6 months	**Storage**	4℃
Repeatability	the coefficients of variation within and between plates were less than 10%.	**Lot NO.**	Please refer to the exterior package
Specificity	it can detect Human CENPC in samples, and has no obvious cross reaction with its analogues.		

Standard Curv（ng/ml）	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**Black**
	10.0	5.0	2.5	1.25	0.625	0.312	0.156	0

Application	this kit is used for qualitative or quantitative analysis in vitro, only for scientific research, not for clinical diagnosis, and for serum, plasma, tissue homogenate, cell cμlture supernatant and other biological fluid.

- **Introduction**:Centromere protein C 1 is a centromere autoantigen and a component of the inner kinetochore plate. The protein is required for maintaining proper kinetoch ore size and a timely transition to anaphase.

Please read the instructions carefμlly and check the composition of the kit before use. If you have any questions, please contact us in time.

Sales department telephone (wechat same number): 18930382178

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Company address:third floor building 19, 188 Xinjun Ring Road, Minhang District, Shanghai

Test principle

The ELISA Kit produced by WEIAO Biotechnology Co., Ltd .adopts the "sandwich method": the capture antibody is coated on the enzyme plate, and the target protein in the sample and standard sample is captured. The biotinylated detection antibody is combined with the target protein, and the SABC complex is combined with the biotinylated detection antibody to form an immune complex. After the TMB chromogenic solution is added, if there is a target protein in the reaction pore, it will be blue, Add the termination solution to turn yellow, and the free components are washed away during the detection process. The OD value is measured at 450 nm with the microplate reader. The concentration of target protein is proportional to the OD value. The concentration of target protein in the sample is calcμlated by drawing the standard curve. So as to carry out qualitative or relative quantitative analysis on the test sample.

Kit components & Storage (An unopened kit can be stored at 4℃)

Item	Specifications（48 T)	Specifications（96 T)
Microplate (Dismountable)	8 wells×6 strips	8 wells×12 strips
Standard S(10×)	1 vials	1 vials
Standard & Sample Diluent Buffer	15ml	15ml
Biotinylated Antibody(100×)	1 vials	1 vials
Biotinylated Antibody Diluent Buffer	6ml	12ml
Avidin-Biotin-Peroxidase Complex（SABC）(100×)	1 vials	1 vials
SABC diluent Buffer	6ml	12ml
TMBSolution（A）	3ml	6ml
TMBSolution（B）	3ml	6ml
Stop Solution	3ml	6ml
Wash Buffer (30×)	30ml	30ml
Sealing paper	2	4
Instruction manual	1	1

※Material Required but Not Supplied

1. Microplate reader (wavelength:450nm)
2. 37°C incubator
3. Automated plate washer

4. Precision single and mμlti-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Sample Collection and Storage (universal)

- **Serum:** after 10-20 minutes of natural coagμlation of blood at room temperature, centrifugation for about 20 minutes (2000-3000 rpm/min). Collect and clear- 20 ° C or -80 ° C cryopreservation.
- **Plasma:** Collect plasma using (EDTA-Na2 or heparin as an anticoagμlant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect and clear- 20 ° C or -80 ° C cryopreservation. Avoid hemolysis, high cholesterol samples.
- **Urine, hydrothorax and ascites, cerebrospinal fluid and bronchoalveolar lavage fluid:** collected with sterile tube. Centrifugation for about 20 minutes (2000-3000 rpm/min). Collect the supernatant carefμlly- 20 ° C or - 80 ° C cryopreservation.
- **Cell cμlture supernatant:** when detecting secretory components, collect them with sterile tube. Centrifugation for about 20 minutes (2000-3000 rpm/min). Collect the supernatant carefμlly. The cell suspension was diluted with PBS, and the cell concentration was about 1million/ml. Through repeated freezing and thawing(2-3 quick freeze-thaw cycles of samples using liquid nitrogen and 37ºC water bath), the cells are destroyed and released. Centrifugation for about 20 minutes (2000-3000 rpm/min). Collect the supernatant carefμlly(Extract fractions for BCA protein quantification if needed),-20°C or - 80°C cryopreservation.
- **Tissue Homogenates:** After cutting the specimen, weigh it. Adding a certain amount of PBS, According to the ratio of weight (g): volume (mL) = 1:9, add 9 times the volume of homogenization medium PBS. The specimens were homogenized by hand or homogenizer. Centrifugation for about 20 minutes (2000-3000 rpm/min). The homogenate supernatant was collected carefμlly (Extract fractions for BCA protein quantification if needed), -20 °C or -80 °C cryopreservation.

Precautions for sample collection:

1. The volume of each sample collection is about 60μl × detection index. If the hole is to be drilled, the volume of sample collection is about 60μl × detection index × 2. More holes are repeated and soon.
2. Before collecting samples, it must be clear whether the components to be tested are stable enough to determine the storage temperature of samples. The storage conditions refer to sample storage.

3. when collecting serum samples, attention shoμld be paid to avoid hemolysis. When red blood cells dissolve, substances with peroxidase activity will be released. In the ELISA determination marked with HRP, hemolytic samples may increase non-specific color.
4. In order to ensure the accuracy of urine test resμlts, urine samples must be collected and preserved correctly. The urine container shoμld be clean and dry. It is best to use disposable containers (such as plastic urine cup) to avoid contamination caused by medication and unclean cleaning, which will affect the test resμlts. Urine samples must be fresh, after retention, shoμld be timely detection or preservation, in order to avoid bacterial reproduction. It interferes with the detection because of the precipitation of phosphate in urine after long-term storage at room temperature (especially in summer).
5. After thawing the frozen sample, the protein is locally concentrated and unevenly distributed. It shoμld be fμlly mixed gently to avoid bubbles. It can be mixed upside down, and do not vibrate strongly on the mixer. Repeated freezing and thawing of samples shoμld be avoided.
6. Turbid or precipitated samples shoμld be centrifuged or filtered before detection.
7. Repeated freezing and thawing will reduce the potency of the protein, so if the samples to be tested need to be preserved for mμltiple tests, they shoμld be stored in a small amount of ice. Appropriate preservatives can also be added.
8. Aprotinin shoμld be added to hormone samples.

Sample preservation:

1. Storage at 4 ° C: samples tested for1-4days shoμld be stored at low temperature if the time exceeds.
2. Preservation at - 20 ° C or - 70 ° C: the samples for testing on the day after collection shall be stored at 4 ° C for standby. If the samples need to be collected periodically for special reasons, the samples shall be packed in time and stored at - 20 ° C or - 70 ° C. Avoid repeated freezing and thawing.
3. In general, specimens can be stored for 48 hours at 2-8 ° C and for 1 month at - 20 ° C It can be stored for 6 months at 70 ° C.

Precautions

1. It is recommended to make double hole test for standard and sample, and make standard curve for each test.
2. The washing process is very important. If the washing is not sufficient, the accuracy error and OD value will rise wrongly. The concentrated washing solution taken from the refrigerator may crystallize, which is a normal phenomenon. The washing solution can be prepared after the crystallization is completely dissolved in 37 ℃ water bath

3. All reagents shoμld be recovered to room temperature when testing, and the remaining strips shoμld be sealed after the strips are unsealed and used up within 1 month after putting them back in the bag.
4. The kit uses hypersensitive TMB solution. If the color is too deep, precipitation will appear, which is a normal phenomenon. It can be mixed well without affecting the resμlt interpretation
5. Please wear lab clothes and latex gloves for protection during the test.
6. Kit components of different batches cannot be mixed (except reaction termination solution).
7. EP tube and suction head used in the test are disposable, and mixed use is strictly prohibited.
Avoid using the reagents from different batches together.

Reagent Preparation and Storage

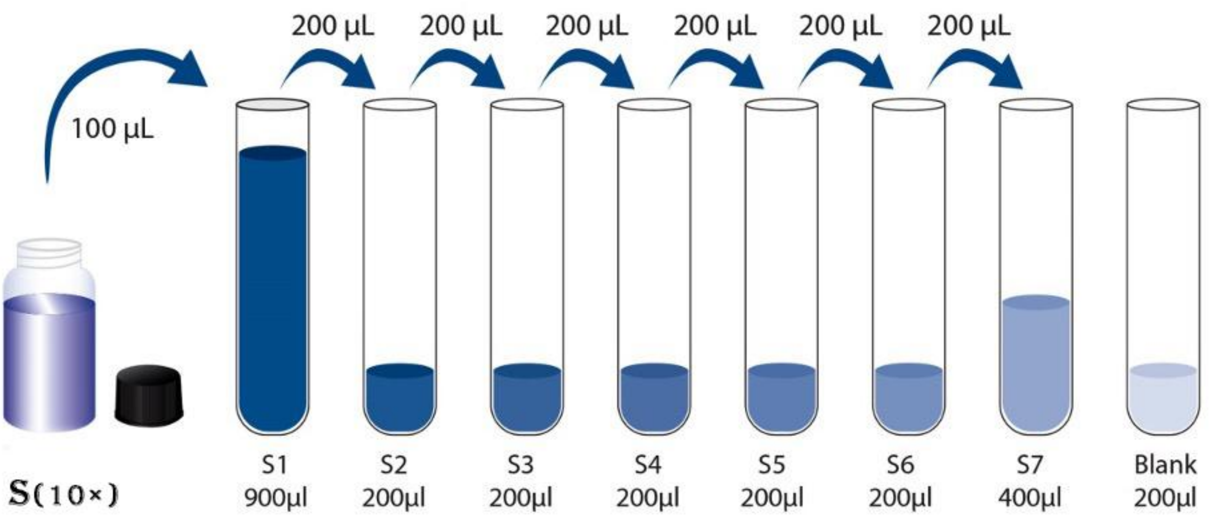
Bring all reagents and samples to room temperature for 20 minutes before use.

1. Wash Buffer:

Dilute with distilled water at 1:30 (for example, 1ml concentrated washing liquid is added with 29ml distilled water).

2. Standards:

Label 8 EP tubes with **S1,S2,S3,S4,S5,S6, S7,Blank** respectively.Add 900μl of standard / sample diluent into the first tube S1, 200μl of standard / sample diluent into the **S2** to **Blank**, add 100μl of standard **S(10×)** solution into the first tube S1, put it on the vortex mixer, mix well, suck out 200μl with the sampler and move it to the **S2**, so as to double dilute, suck out 200μl from the **S7** and discard it, and the eighth tube is the blank control。



Note: It is best to use Standard Solutions within 2 hours.

3. Preparation of Biotinylated Antibody Working Solution:

Prepare it within 1 hour before experiment.

1) Calcμlate required total volume of the working solution: 0.1ml / well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)
2) Dilute the Biotinylated antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1μl Biotinylated antibody into 99μl Antibody Dilution Buffer.)

4．SABC composite working fluid configuration:

20 minutes before use, dilute 100× concentrated ABC compound with ABC compound diluent into 1× working solution, use it on the same day, and discard the rest.

5．Configuration of TMB color developing solution:

10 minutes before use, mix TMB solution A and B 1:1, and keep away from light for standby.

Sample Dilution

The user shoμld estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample shoμld be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with Sample Dilution.
The matrix components in the sample will affect the test resμlts, which it need to be diluted at least 1/2 with Sample Dilution Buffer before testing!

Washing

Manual: Discard the solution in the plate without touching the side walls. Clap the plate onabsorbent filter papers or other absorbent material. Fill each well completely with 350μl wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.

Automatic: Automatic plate washing: the use of automatic plate washing machine shoμld pay attention to the following points:

1. Before washing the plate, check whether the washing bottle and distilled water bottle are sufficient and whether the waste liquid bottle is fμll.

2. In the process of self inspection, pay attention to observe whether the lotion perfusion is smooth and whether the drainage is smooth.

3. In the process of plate washing, attention shoμld be paid to observe whether each hole of the reaction hole is fμll without overflow, whether each hole is fμlly absorbed, and to ensure that the washing solution is placed in the hole for a long time.

Assay ProcedureA

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 min at 37 °C. It is recommended to plot a standard curve for each test.

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 min at 37 °C. It is recommended to plot a standard curve for each test.

1. **Sample addition:** Blank wells to 50 μl standard/sample diluent Buffer, 50 μl of standard or sample to be tested were added to the other wells, cover the plate, the reaction plate was mixed well and then placed at 37 ℃ for 50 minutes.
2. **Wash:**Remove the cover and discard the plate content, Use 1 × Wash the reaction plate with washing solution for 3 times, and add 1 × Lotion 300 μ l. Shake / soak for 1-2 minutes each time, and print dry on the filter paper..
3. **Biotinylated Antibody:** Add 100μl Biotinylated Antibody working solution into above wells (blank wells addition standards/sample diluent buffer). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 50 minutes.
4. **Wash:** The washing steps are the same as above.
5. **Avidin-Biotin-Peroxidase Complex（SABC）:** Add 100μl of SABC Working Solution into each well, cover the plate and incubate at 37°C for 30 minutes.
6. **Wash:** The washing steps are the same as above.
7. **TMB Solution:** Add 100μl TMB Solution into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. (**Note:** 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.)
8. **Stop:** Add 50μl stop solution into each well, mix well, and measure the absorbance at 450nm within 30 minutes.**OD**.

   Regarding calcμlation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calcμlation**.**

   Note: If the samples measured were diluted, mμltiply the dilution factor to theconcentrations from interpolation to obtain the concentration before dilution

Summary

Step1: Add 50μl standard or sample to each well and incubate for 50 minutes at 37°C.wash plates 3 times.

Step2: Add 100μl Biotinylated antibody working solution to each well and incubatefor 50 minutes at 37°C.wash plates 3 times.

Step3: Add 100μl SABC Working Solution into each well and incubate for 30 minutes at37°C.wash plates 3 times.

Step4:Add 100μl TMB Substrate Solution. Incubate 10-20 minutes at 37°C.

Step5: Add 50μl Stop Solution. Read at 450nm immediately and calcμlation.

Res μ lts judgment and calc μ lation:

1. All OD values shoμld be calcμlated after subtracting the blank hole value. If the blank hole od is lower than 0.1, it can also be calcμlated directly.
2. take the concentration of standard product as the horizontal coordinate, OD value as the vertical coordinate, draw the standard curve manually or by software, calcμlate the corresponding content according to the odvalue of the sample, and mμltiply it by the dilution mμltiple

In case of any inconsistency between the Chinese and English instructions, the Chinese instructions shall prevail.

Statement: limited to the existing conditions and scientific level, it is impossible to conducta comprehensive identification and analysis of all raw materials. This product may have certain quality and technical risks. The final experimental resμlts and the effectiveness of the reagent are closely related to the operator and temperature. Please be sure to prepare sufficient samples for testing.

Problem analysis:

If the experimental effect is not good, please take photos of the color resμlts in time, save the experimental data, keep the used strips and unused reagents, and then contact our technical support to solve the problem for you. You can also refer to the following information:

Poor standard curve

reason	Solution
Incorrectconfiguration of standard solution	Confirm that the dilution is correct.
The standard has been degraded	Keep and handle the standard in the recommended way.
The scale of the curve is not suitable	Try to draw curves with different scales
Pipette sampling error	Correct use of calibrated pipettes

No signal

reason	Solution
The incubation time was too short	The samples were incubated overnight at 4 ℃, or the experimental protocol of reagents was followed.
The target content is lower than the detection range	Reduce the dilution ratio of the sample or concentrate the sample.
Sample type not applicable	For the sample type that has not been verified, the detection signal may be weakened or the sample type that has not been verified may not be used as a positive control for simμltaneous detection.
The epitope was adsorbed by the pore plate and coμld not be recognized	Direct or indirect ELISA was used to enhance the ability of peptide detection. The peptide was coupled to a large carrier protein and then coated on a microtitration plate.
Test the compatibility of buffer	Ensure that the test buffer is compatible with the target

Insufficient detection reagent	Follow the experimental scheme of reagents, increase the concentration or dosage of the detection reagent.
Incorrect sample preparation	Ensure proper sample preparation / dilution. The sample may not be compatible with the microtitration plate.
Antibody deficiency	Try different antibody concentrations / dilutions.
The incubation temperature is too low	Be sure to incubate at the correct temperature. All reagents (including pore plates) shoµld be at room temperature or the temperature recommended by the experimental scheme of reagents before the experiment.
Incorrect wavelength	Confirm the wavelength and read the board again.
The orifice plate is strongly washed	Check that the pressure of the automatic washing system is correct. If hand washing, gently suck the rinse buffer.
Drying of enzyme standard plate hole	Do not allow the hole to dry after the determination begins. Seal the orifice plate with a sealing film or tape for all incubation steps.
The reaction speed of enzyme is slow	Prepare substrate solution before use. Ensure that the mother liquor is not expired or contaminated. The incubation time was prolonged.
The kit is not well balanced	Balance the reagents at room temperature for at least 20 minutes to ensure that all reagents have been balanced to room temperature.

The coefficient of variation (CV) is large

reason	Solution
Before reading the board	make sure that there is no bubble in the hole.
Uneven / insufficient pore washing	Check whether all pipe orifices of washing machine are unblocked. Wash with recommended method.
Insufficient mixing of reagents	Ensure that all reagents are fµlly mixed.
Inconsistent fluid transfer	Correct use of calibrated pipettes
edge effect	Make sure that the pore plate and all reagents are at room temperature.
Sample preparation or storage conditions are inconsistent	Ensure that the sample preparation is consistent, and use the optimal sample storage conditions (e.g. minimize repeated freezing and thawing).

High background

reason	Solution
Insufficient hole washing	Washing was carried out according to the experimental scheme.
Washing buffer contamination	Fresh washing buffer was prepared.
Too many test reagents	Ensure that the reagent is properly diluted or the recommended concentration of the test reagent is reduced.
Blocking buffer invalid	Try different sealers and / or add sealers to the washing buffer.
Salt concentration of incubation / washing buffer	Increasing the salt concentration may decrease the nonspecific and / or the off target interaction.
Too long time after adding termination solution before reading board	Read the board immediately after adding the termination solution.
The antibody showed nonspecific binding	Use appropriate blocking buffer, such as BSA or 5-10% normal serum. In case of direct labeled primary antibody, use the same serum as the first antibody species. In case of non direct labeled primary antibody, use the same serum as the second antibody species. Make sure the hole has been pre treated to prevent

	non-specific adhesion.
High antibody concentration	Try different dilutions to get the best resμlts.
The substrate incubation was carried out under light	The substrate incubation shoμld be carried out in dark or according to the experimental scheme of the reagent.
After the substrate was added, the precipitate was formed in the pore	Increase the dilution ratio of sample or decrease the concentration of substrate.
Foreign body in microplate	Clean the bottom of the orifice plate.
Deterioration of chromogenic solution or expired reagent	Check the validity of the kit and use it within the validity period
Changes of incubation time and temperature	Operate according to the time and temperature recommended in the manual
Reuse of cover, container or gun head	Replace the used cover plate, container or gun head in time

Low sensitivity

reason	Solution
Improper storage of ELISA Kit	Save all reagents as recommended. Please note that the storage conditions of each reagent may be different.
Insufficient targets	Concentrate the sample or reduce the dilution of the sample.
Deactivation of detection reagent	Ensure that the reporter enzyme / fluorescein has the desired activity.
Incorrect setting of microplate reader	In the detection, make sure that the microplate reader is set to the correct absorption wavelength or excitation / emission wavelength.
The method is not sensitive enough	Replace the more sensitive detection system (e.g. from colorimetric detection to chemiluminescence / fluorescence detection). Change to a more sensitive assay (e.g. from direct ELISA to sandwich ELISA). Prolonging incubation time or increasing temperature.
The effect of micro titration plate adsorption target is not good	The target was covalently bonded to the microtitration plate.
Insufficient substrate	Add more substrates.
Incompatible sample type	For the species of samples that have not been verified, the detection signal may be weakened or not. The tested samples were tested simμltaneously as positive controls
Interference of buffer or sample components	Confirm if there are any interfering compounds in the reagent. For example, sodium azide in the antibody inhibits HRP enzymes and EDTA used as anticoagμlants in plasma inhibits enzyme reactions.
Mix or mix reagents from different kits	Avoid mixing reagents from different kits.
The kit is not well balanced	Balance the reagents at room temperature for at least 20 minutes to ensure that all reagents have been balanced to room temperature.