# Canine CA9 Elisa Kit

- 检测范围：15.6－1000pg/ml
- 灵 敏 度：＜2.84pg/ml
- 标准曲线对应浓度:(pg/ml)

| S1 | S2 | S3 | S4 | S5 | S6 | S7 | blank |
|---|---|---|---|---|---|---|---|
| 1000.0 | 500.0 | 250.0 | 125.0 | 62.5 | 31.25 | 15.6 | 0 |

- 规　格：48T/96T
- 保　存：4℃
- 有效期：6 个月
- 特异性：系统和其它类似物无明显交叉反应。
- 精密度：板内变异系数均＜9%，板间变异系数均＜10%
- 用　途：适用于检测血清、血浆、组织匀浆、细胞培养上清、细胞因子等样品。
  其它样本类型请咨询技术支持。
- 简介：碳酸酐酶 9（CA9/CAIX）是一种由 CA9 基因编码的酶。碳酸酐酶（CAs）是锌金属酶的一个大家族，催化二氧化碳的可逆水合作用。它们参与多种生物过程，包括呼吸、钙化、酸碱平衡、骨吸收以及房水、脑脊液、唾液和胃酸的形成。该基因定位于 9p13.3。已知与碳酸酐酶 a 和碳酸酐酶 C 相关的蛋白质。在所有透明细胞肾癌中均有表达，但在正常肾脏或大多数其他正常组织中未检测到。它可能参与细胞增殖和转化。

**本试剂盒仅供科学研究使用，不用于临床诊断！使用前务必仔细阅读说明书！**

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:

销售部电话：18968009509，18968000935，18969978509

技术部电话：0571-86733691

邮箱(技术部)：826710510@qq.com

网址： www.jinhengnuo.com

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。

**检测原理：**

本试剂盒采用双抗体“夹心法”，把捕获抗体包被于酶标板上，加入待检样品及标准品于酶标板反应孔中、捕获抗体捕获对应目的蛋白，生物素标记的检测抗体与目的蛋白结合，检测抗体再和 SABC 结合，形成捕获抗体-目的蛋白-检测抗体-SABC 复合物，经过洗液洗涤后，未结合的成分均被洗去，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，颜色的深浅和样品中的目的蛋白浓度呈正相关，通过绘制标准曲线计算出样品中目的蛋白的浓度，从而进行定性或半定量分析。

**试剂盒组分：**

| 组分 | 规格 | |
|---|---|---|
| | 48T | 96T |
| 酶标板（Coated Wells） | 8 孔×6 条 | 8 孔×12 条 |
| 标准品（S1）(Standard S1) | 1 支 | 1 支 |
| 标准品/样品稀释液(Standard/Sample Diluent ) | 6ml | 12ml |
| 生物素标记检测抗体（100×）(Concentrated Biotin Antibody 100× ) | 60ul | 120ul |
| 生物素标记检测抗体稀释液(Concentrated Biotin Antibody Diluent) | 6ml | 12ml |
| SABC（100×）(Strept avidin-biotin complex 100× ) | 60ul | 120ul |
| SABC 稀释液(Strept avidin-biotin complex Diluent ) | 6ml | 12ml |
| TMB 显色液（A/B）(TMB Chromogen A/B) | 6ml | 12ml |
| 终止液(Stop Solution) | 3ml | 6ml |
| 30×洗涤缓冲液(30×Wash Buffer) | 25ml | 25ml |
| 封板膜(Plate Sealer) | 2 | 4 |

**试剂盒的储存及有效期：**

1、未拆封的试剂盒 4℃保存，6 个月有效。

2、拆封后的试剂盒，请将未使用酶标条用自封袋装好， 1 个月内有效。

**提示：**

- 使用前请检查试剂盒中试剂的标签和数量与表格是否一致，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，请在使用时量取使用而非直接倒出使用。

**样品的收集和保存：**

1. 血清样品:将收集于血清分离管中的全血标本在室温放置 2 小时或 4℃ 过夜，然后 1,000×g 离心 15 分钟，取上清即可，将上清置于≤-20℃冷冻保存，避免反复冻融。
2. 血浆样品:用 EDTA 或肝素钠抗凝管采集标本，并将标本在采集后的 30 分钟内于 4℃冰箱，1,000×g 离心 15 分钟， 取上清即可检测，或将上清置于≤-20℃冷冻保存，避免反复冻融。
3. 组织匀浆:
   A、在预冷 PBS(0.01mol/L， pH=7.0-7.2)中清洗去除血液，低温切割标本后，准确称取组织重量。

   B、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充分匀浆。

   C、3000 ×g 离心 15 分钟，仔细收集匀浆上清，弃沉淀，（如需要可取部分进行 BCA 蛋白定量），≤-20℃以下保存。

4. 细胞裂解液:在分析试验之前，细胞需利用以下方法处理:
   A、贴壁细胞应该用冷 PBS 轻轻清洗，然后用胰蛋白酶消化，于 1,000×g 离心 5 分钟后收集，(悬浮细胞通过离心直接收集)，将收集到的细胞用冷 PBS 洗 3 次。

   B、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方式，以使细胞破坏并放出细胞内成份。3000 ×g 离心 15 分钟，仔细收集上清，≤-20℃冷冻保存。

5. 细胞培养上清或其它生物体液标本:请 3,000×g 离心 15 分钟，取上清即可检测，或将上清置于≤-20℃冷冻保存，避免反复冻融。

**提示:**

- 标本均需密封保存，4℃保存不超过 1 周，≤-20℃保存不超过 1 个月。
- 标本出现溶血会影响最后检测结果，故溶血标本不宜进行 ELISA 检测。

**样本稀释原则**

使用者应估计样品待测因子的含量，来决定是否对样本进行适当的稀释检测，以便使样品中目的蛋白的浓度处于本试剂盒的最佳检测范围内。

参考稀释方案如下:
待测样本目的蛋白含量超低的----浓缩后检测。

待测样本目的蛋白含量低的----直接原液检测。

待测样本目的蛋白含量中的----稀释后检测。一般按 1:10 稀释，270ul 稀释液加 30ul 样品。

待测样本目的蛋白含量高的----稀释后检测。一般按 1:100 稀释，297ul 稀释液加 3ul 样品。

待测样本目的蛋白含量超高的----稀释后检测。一般按 1:1000-10000 稀释。

**样品 1000 倍稀释:**分两步稀释。取 5ul 样本移至 95ul 稀释液内为 A 液，为 20 倍稀释，再取 A 液 5ul 移至 245ul 稀释液内，为 50 倍稀释， 总共稀释 1000 倍。
**样品 10000 倍稀释:**分三步稀释。取 5ul 样本到 195ul 稀释液内为 A 液，40 倍稀释；再取 A 液 5ul 移至 245ul 稀释液内为 B 液，50 倍稀释； 最后取 B 液 60ul 移至 240ul 稀释液内，为 5 倍稀释，总共稀释 10000 倍。

血清、血浆、灌洗液、尿液、胸水、唾液等体液建议原液检测（个别指标除外），以上方案仅供参考，最好做预实验，以确定稀释倍数，样品的稀释应有详细记录。

**需自备的设备及试剂：**
1、酶标仪（含 450nm 滤光片）， 使用前仪器提前预热。
2、单道及多道微量移液器及无菌吸头。
3、1.5ml 离心管。
4、蒸馏水或去离子水。
5、吸水纸。
6、量筒烧杯等容器

**洗板方法**

手工洗板方法:

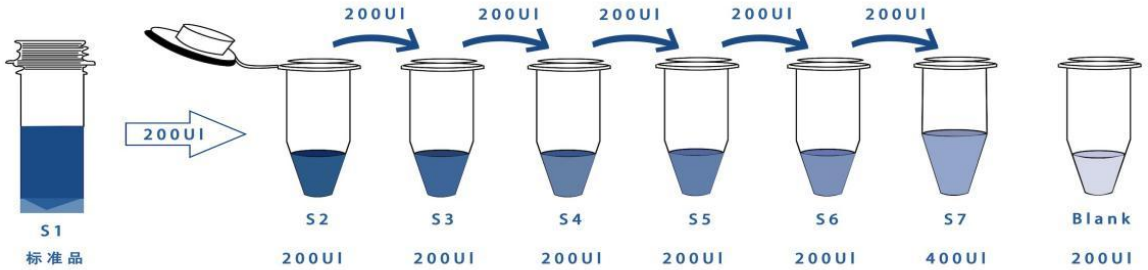
吸去或甩掉酶标板内的液体，在实验台上铺垫几层吸水纸， 酶标板朝下用力拍几次，每孔加入 1×洗涤缓冲液 300ul，浸泡 1-2 分钟，重复此过程数次。

自动洗板:

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。

**测前准备**

1、实验测试前 30 分钟，将所有的试剂及样品平衡至室温，不能加热使之融解，已经倒出的试剂请勿倒回瓶中，避免瓶中试剂污染，试剂配置或样品稀释时，切记要混匀。
2、每次检测都应该做标准曲线，使用者应估计样品待测因子的含量，来决定是否对样本进行适当的稀释检测，以便使样品中目的蛋白的浓度处于本试剂盒的最佳检测范围内。
3、当待检样品需要稀释时，如标准品/样品稀释液不够用，可以用 PBST 替代，请提前准备 PBST。
4、洗涤液配置（1×）：用纯水 1:30 稀释浓缩洗涤液(1ml 浓缩洗涤液加入 29ml 的纯水)。当稀释液及洗涤液不够用时，可以用 1*PBST 替代，洗涤液中如有结晶析出，请先温育至室温，轻轻混匀，至到结晶完全溶解再进行配制，配置过程请使用纯水，避免因水污染导致实验失败。
5、抗体工作液配置：100ul 生物素检测抗体（100×）加入 10ml 抗体稀释液中。
6、SABC 工作液配置：100ul SABC（100×）加入 10mlSABC 稀释液中。
7、TMB 显色液的配置:使用前 5 分钟，将 TMB 显色液 A 液和 B 液 1:1 混合， 避光放置备用，在储存和显色时均避免强光照射。
8、标准品配置：取 7 个 1.5ml 离心管，分别标注：S2、S3、S4、S5、S6、S7、blank，每管中分别加入标准品/样品稀释液 200ul，从试剂盒中取出标准品溶液（S1），用移液器吸出 200ul，移至第二管 S2 中， 置于混合器上混匀后用移液器吸出 200ul 移至第三管 S3 中，如此反复作对倍稀释至 S7，标准品/样品稀释液为空白对照孔 blank，标准品共 8 个孔，即：S1、S2、S3、S4、S5、S6、S7、blank，当日使用，剩余弃之。




**检测流程：**

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 50ul，将酶标板用封板膜封好，轻轻混匀后置 37℃，孵育 50 分钟。
2. **洗　　板**：用洗涤液（1×）将酶标板充分洗涤 3 次，每孔加入洗液 300μl，每次震荡/浸泡 1-2 分钟，向滤纸上印干。
3. **孵育抗体**：空白孔加入 100ul 的抗体稀释液，其余孔各加入检测抗体工作液 100ul，将酶标板用封板膜封好，轻轻混匀后置 37℃，孵育 50 分钟。
4. **洗　　板**：同上。
5. **孵育 SABC**：空白孔加入 100ul 的 SABC 稀释液，其余孔各加入 SABC 工作液 100ul，将酶标板用封板膜封好，轻轻混匀后置 37℃，孵育 30 分钟。
6. **洗　　板**：同上。
7. **显　　色**：每孔加入提前配置好的 TMB 混合液 100ul，将酶标板用封板膜封好， 轻轻混匀后置 37℃，暗处反应 8-20 分钟，反应结果为蓝色。

8. **终止反应：**每孔加入 50ul 终止液，轻轻混匀，此时蓝色转为黄色，20 分钟内用酶标仪在 450nm 处测 OD 值。

**提示：**

- 加样:实验操作中请使用一次性的吸头，避免交叉污染。加样时注意不要有气泡，将样品加于酶标板底部，尽量不触及孔壁，轻轻晃动混匀。加样或加试剂时，第一个孔与最后一个孔加样之间的时间间隔如果太大， 将会导致不同的“孵育”时间，从而明显地影响到测量值的准确性及重复性。因此，一次加样时间(包括标准品及所有样品)最好控制在 10 分钟内。
- 孵育:为防止样品蒸发，实验时请将封板膜封好，以避免液体蒸发，洗板后应尽快进行下步操作，任何时侯都应避免酶标板处于干燥状态，同时应严格遵守孵育时间和温度。
- 洗涤:充分的洗涤非常重要，在每次洗涤过程中，都要将洗涤液完全甩干，洗涤过程中反应孔中残留的洗涤液应在滤纸上拍干，勿将滤纸直接放入反应孔中吸水，同时要消除板底残留的液体和手指印，避免影响最后的酶标仪读数。
- 显色时间的控制: 说明书中显色时间供参考，因用户实验室条件差异，最佳显色时间会有所不同，加入底物后请定时观察反应孔的颜色变化(比如，每隔 5 分钟观察一次)，如颜色较深，请提前终止反应。当标准曲线有明显梯度且 S7 孔肉眼可见淡淡的蓝色时便可终止反应，避免反应过强从而影响酶标仪光密度读数。
- 本试剂盒中使用了酸作为终止液，具有腐蚀性，使用时应避免接触衣物或眼、手等皮肤暴露部位。

**结果判断与计算:**

1. 空白孔设为对照孔，所有的标准品和样品的吸光值减去空白孔的后，得到的数据可以直接在坐标纸上画出曲线，如空白孔吸光值（OD）值低于 0.1 时，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。
3. 如 S1 检测 OD 值超出酶标仪检测范围时，可以舍弃其值进行统计分析，不影响实验结果。

**回收率：**

分别于血清及血浆样本中加入已知蛋白，重复测定并计算其均值，回收率为测定值与理论值的比率，通过测试均在回收范围内。

| 样本 | 回收率范围(%) |
|---|---|
| 血清 | 88-96 |
| EDTA 抗凝血浆 | 87-96 |
| 肝素钠抗凝血浆 | 83-95 |

**线性范围：**

在血清及血浆样本中加入一定量的目的蛋白，并倍比稀释待测样本，线性范围即为稀释后样本中目的蛋白含量的测定值与理论值的比率。

**精密度：**

精密度用样品测定值的变异系数 CV 表示。CV(%) = SD/mean×100

SD 值是标准差（Standard Deviation），是离均差平方的算术平均数的算术平方根，用 σ 表示,标准差也被称为标准偏差，或者实验标准差。

批内差:取同批次试剂盒对低、中、高值定值样本进行定量检测,每份样本连续测定 20 次，分别计算不同浓度样本的平均值及 SD 值，批内差: CV<9%。

批间差:选取 3 个不同批次的试剂盒分别对低、中、高值定值样本进行定量测定，每个样本使用同一试剂盒重复测定 8 次，分别计算不同浓度样本的平均值及 SD 值，批间差: CV<10% 。

**稳定性：**

经测定，试剂盒在有效期内按推荐温度保存，其活性降低率小于 5%。 为减小外部因素对试剂盒破坏前后检测值的影响，实验室的环境条件需尽量保持一致，尤其是实验室内温度、及温育条件,其次由同一实验员来进行操作可减少人为误差。

**问题分析：**

如实验结果不理想，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，填写售后服务表格（网上下载填写），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：

**标准曲线差：**

| 可能原因 | 相应对策 |
|---|---|
| 标准品溶液配置有误 | 吸液或加液不准 ，检查移液器及吸头 ，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。 |
| 洗涤不完全 | 保证洗涤时间和洗涤次数及每孔的加液 |
| 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：**

| 可能原因 | 相应对策 |
|---|---|
| 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。 |
| 检测缓冲液的相容性 | 确保检测缓冲液与靶标兼容 |
| 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 | 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。 |

**变异系数大：**

| 可能原因 | 相应对策 |
|---|---|
| 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。 |
| 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：**

| 可能原因 | 相应对策 |
|---|---|
| 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 | 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/或减弱脱靶相互用。 |

| | |
|---|---|
| 读板前加入终止液后时间太长 | 添加终止液后立即读板。 |
| 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。 |
| 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。 |
| 封板膜重复使用 | 及时更换使用过的封板膜。 |

**灵敏度偏低：**

| 可能原因 | 相应对策 |
|---|---|
| ELISA 试剂盒保存不当 | 按推荐方式保存所有试剂。 |
| 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。 |
| 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。 |
| 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。 |
| 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。 |

**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。最终的实验结果与试剂的有效性、实验者的相关操作以及实验环境密切相关。
2、由于现有条件及科学技术水平尚不能对供货商提供的所有原料进行全面的鉴定与分析，本产品可能存在一定的质量技术风险，如实验失败，使用者自己承担风险，公司不承担试剂盒以外的任何实验失败损失。
3、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。
4、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。
5、细胞培养上清样品，因该类样本干扰因素较多，包括细胞状态、细胞活力，细胞数量，以及采样时间等因素，所以可能存在检测不出的情况。
6、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。
7、不同批次的同一产品可能会有少许差别，如:检测限、灵敏度以及显色时间等，请依据试剂盒内说明书为准，网站电子版说明书仅作参考。

8、只有全部使用试剂盒中的试剂才能保证检测效果，不能混用其他制造商的产品，只有严格遵守本试剂盒的实验说明才会得到最佳的检测结果。
9、在储存运输过程中避免将试剂暴露在强光中，所有试剂瓶盖须盖紧以防止蒸发和微生物污染，导致试剂失效或污染而结果不准确。
10、刚开启的酶标板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，酶标板在使用时从包装袋里取出，请勿提前取出。
11、在样本制备以及操作的每个过程中的变化都可能导致不同的实验结果，所以为了提高实验结果的可重复性，实验的每一步操作都需要严格控制。
12、试剂盒在出厂前均经过严格质检，但由于运输条件及各实验室条件差异，可能会造成实验结果与出厂结果不一致或不同批次试剂盒批间差增大的情况。
13、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蛋白的产品做对比，所以不排除检测结果不一致的情况。
14、用于制备试剂盒中抗体的免疫原通常为重组蛋白，但由于制备重组蛋白所选取的片段、表达系统、纯化方式等各有不同，所以我们无法保证该试剂盒可用于其他公司重组蛋白的检测。
15、该试剂盒可能不适用于一些实验本身有效性不确定的特殊实验样品的检测，例如，基因敲除实验等样品。
16、该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。

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# Canine CA9 Elisa Kit

- Detection range: 15.6 - 1000pg/ml
- Sensitivity: ＜2.84pg/ml
- The standard curve corresponds to the concentration:(pg/ml)

| S1 | S2 | S3 | S4 | S5 | S6 | S7 | blank |
|---|---|---|---|---|---|---|---|
| 1000.0 | 500.0 | 250.0 | 125.0 | 62.5 | 31.25 | 15.6 | 0 |

- Specifications: 48T/96T
- Preservation: 4℃
- Shelf life: 6 months
- Specificity: There is no significant cross-reaction between the system and other analogues.
- Precision: Average in-board coefficient of variation <9%, inter-board coefficient of variation <10%.
- Usage: It is applicable to the detection of serum, plasma, tissue homogenate, cell culture supernatant, cytokine and other samples. For other sample types, please consult Technical Support.
- Introduction: Carbonic anhydrase 9(CA9/CAIX) is an enzyme that in humans is encoded by the CA9 gene. Carbonic anhydrases(CAs) are a large family of zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide. They participate in a variety of biological processes, including respiration, calcification, acid-base balance, bone resorption, and the formation of aqueous humor, cerebrospinal fluid, saliva, and gastric acid. This gene is mapped to 9p13.3. CA IX is a transmembrane protein and the only tumor-associated carbonic anhydrase isoenzyme known. It is expressed in all clear-cell renal cell carcinoma, but is not detected in normal kidney or most other normal tissues. It may be involved in cell proliferation and transformation.

**This kit is for scientific research purposes only and is not intended for clinical diagnosis! Be sure to read the instructions carefully before use!**

Please read the instruction carefully before use, if you have any questions, please contact us through the following ways:

Sales Department Tel: 18968009509, 18968000935, 18969978509

Technical Department Tel: 0571-86733691

E-mail (Technical Department): 826710510@qq.com

Website: www.jhnbio.com

For specific shelf life, please refer to the outer packaging label of the kit, please use the kit during the shelf life.

Detection principle:

The kit adopts the double antibody "sandwich method", the capture antibody is coated on the enzyme plate, the sample to be examined and the standard are added to the reaction well of the enzyme plate, the capture antibody captures the corresponding protein of interest, the detection antibody labeled with the enzyme of interest is combined with the protein of interest, forming a complex of the detection antibody of the capture antibody - protein of interest - horseradish peroxidase label, after washing the liquid, the unbound components are washed off, and the color-developing substrate TMB is added, and the TMB is blue under the catalysis of horseradish peroxidase. It turns yellow after adding the stop solution. The OD value was measured at a wavelength of 450 nm with a microplate reader, the shade of color was positively correlated with the concentration of the protein of interest in the sample, and the concentration of the protein of interest in the sample was calculated by plotting a standard curve for qualitative or semi-quantitative analysis.

Components of the kit:

| Components | Specification | |
|---|---|---|
| | 48T | 96T |
| Coated Wells | 8wells×6strip | 8wells×12strip |
| Standard S1 | 1 支 | 1 支 |
| Standard/Sample Diluent | 6ml | 12ml |

| | | |
|---|---|---|
| Concentrated Biotin Antibody 100× | 60ul | 120ul |
| Concentrated Biotin Antibody Diluent | 6ml | 12ml |
| Strept avidin-biotin complex 100× | 60ul | 120ul |
| Strept avidin-biotin complex Diluent | 6ml | 12ml |
| TMB Chromogen A/B | 6ml | 12ml |
| Stop Solution | 3ml | 6ml |
| 30×Wash Buffer | 25ml | 25ml |
| Plate Sealer | 2 | 4 |

**Storage and expiration date of the kit:**

1、Unopened kits are stored at 4 ° C and are valid for 6 months.

2、After unpacking the kit, pack the unused microplate strip in a self-sealing bag, which is valid for 1 month.

**Prompt:**

- Before use, please check whether the label and quantity of reagents in the kit are consistent with the table, all reagent bottle caps must be tightened to prevent evaporation and microbial contamination, the volume of reagents is subject to the actual shipping version of the instructions, the relevant reagents will be slightly more than the volume indicated on the label when packing, please take the amount of use during use rather than directly pouring out.

**Collection and preservation of samples:**

1. Serum samples: Place whole blood samples collected in serum isolation tubes at room temperature for 2 hours or at 4° C overnight, then centrifuge 1,000 ×g for 15 minutes, take the supernatant, and freeze-store the supernatant at ≤ -20° C to avoid repeated freeze-thawing.
2. Plasma samples: Specimens are collected with EDTA or heparin sodium anticoagulant tubes and the specimens are centrifuged in a 4° C freezer, 1,000 ×g for 15 minutes within 30 minutes after collection, and the supernatant can be detected by taking the supernatant, or the supernatant is cryopreserved at ≤-20 ° C to avoid repeated freeze-thawing.
3. Tissue homogenization:

A. Wash and remove blood in pre-chilled PBS (0.01mol/L, pH = 7.0-7.2), and accurately weigh the tissue weight after cutting the specimen at low temperature.

B. According to the ratio of weight (g): volume (mL) = 1:9, add 9 times the volume of homogeneous medium PBS. Homogenize the specimens thoroughly by hand or with a homogenizer.

C. Centrifuge 3000 × g for 15 min, carefully collect the homogenized supernatant, discard the pellet, (if necessary for BCA protein quantification of the optional part), ≤-20 ° C or less.

4. Cell lysate: Before the analysis test, the cells need to be treated using the following methods:

A. Adherent cells should be gently washed with cold PBS, then tripsinized, collected after centrifugation of 1,000 × g for 5 minutes, (suspension cells are collected directly by centrifugation), and the collected cells are washed 3 times with cold PBS.

B. Dilute the cell suspension with PBS, and the cell concentration reaches about 1 million/ml. By ultrasonic disruption or repeated freeze-thawing, the cells are destroyed and released into the cell components. Centrifuge 3000 × g for 15 min, collect the supernatant carefully and cryopreserve ≤-20 °

5. Cell culture supernatant or other biological fluid specimens: please centrifuge 3,000 ×g for 15 minutes, take the supernatant to detect, or place the supernatant in ≤-20 ° C for cryopreservation to avoid repeated freeze-thaw.

**Prompt:**

- Specimens should be stored sealed, stored at 4 ° C for no more than 1 week, ≤ -20 ° C for no more than 1 month.
- Hemolysis of the specimen will affect the final test result, so the hemolysis specimen should not be tested for ELISA.

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**Sample dilution principles:**

The user should estimate the amount of factors to be measured in the sample to determine whether to perform the appropriate dilution test on the sample so that the concentration of the protein of interest in the sample is within the optimal detection range of this kit.

**The reference dilution protocol is as follows:**

Ultra-low protein content of the sample to be measured----tested after concentration.

The sample to be tested with a low protein content of interest ---- direct stock solution detection.

The protein content of interest in the sample to be measured ---- detected after dilution. Typically diluted 1:10, 270ul dilution plus 30ul sample.

The sample to be tested with a high protein content of interest ---- tested after dilution. Dilute typically at 1:100, 297ul dilution plus 3ul sample.

The sample to be tested has a high protein content of interest---- tested after dilution. Generally diluted at 1:1000-10000.

**Sample 1000-fold dilution:**Dilute in two steps. Take 5ul of samples and move to 95ul dilution for 20x dilution, and then take 5ul of A and 5ul to 245ul dilution for a total dilution of 1000x.

**Sample 10,000-fold dilution:**Dilute in three steps. Take 5ul of samples into 195ul dilution for A solution, diluted 40 times; Then take A solution 5ul and move it to the 245ul dilution to be B solution, diluted 50 times; Finally, take 60ul of B solution and move it into a 240ul dilution for a 5-fold dilution, for a total dilution of 10,000-fold.

Serum, plasma, lavage fluid, urine, pleural fluid, saliva and other body fluids are recommended for the detection of the original solution (except for individual indicators), the above scheme is for reference only, it is best to do pre-experiments to determine the dilution multiple, and the dilution of the sample should be recorded in detail.

**Equipment and reagents to be brought by yourself:**

1、Microplate reader (with 450 nm filter), preheat the instrument in advance before use.
2、Single- and multi-channel micropipettes and sterile tips.
3、1.5 ml centrifuge tube.
4、Distilled or deionized water.
5、Absorbent paper.
6、Measuring cylinder beakers and other containers.

**Washing method**

Hand washing method:

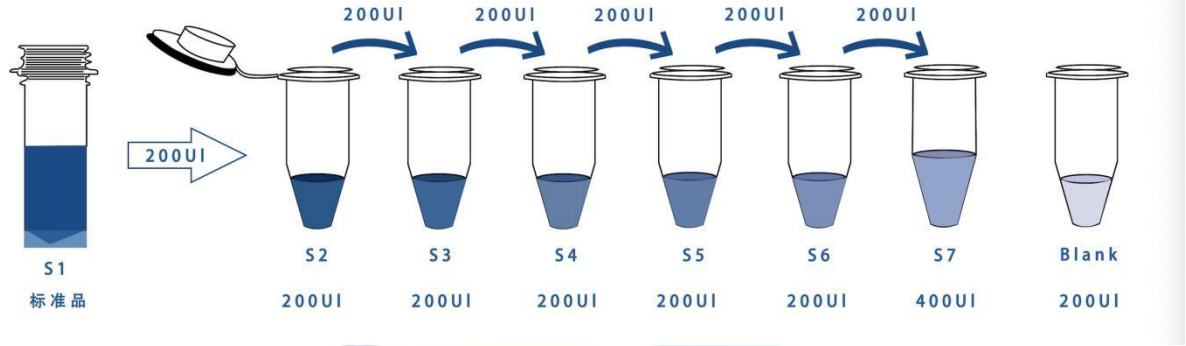
Aspirate or shake off the liquid in the enzyme plate, lay several layers of absorbent paper on the experimental table, pat the enzyme plate down several times, add 1× wash buffer 300ul per well, soak for 1-2 minutes, and repeat this process several times.

Automatic washing:

1. Before washing the plate, check whether the lotion bottle and distilled water bottle are sufficient, and whether the waste liquid bottle is full.
2. During the self-examination process, pay attention to whether the well-perfusion of the lotion is smooth and whether the discharge is unobstructed.
3. During the washing process, attention should be paid to observing whether each well of the reaction well is filled and there is no spillage, whether the water absorbed by each well is exhausted, and the time that the lotion is placed in the well should be ensured.

## Pre-test preparation

1. 30 minutes before the experimental test, all reagents and samples are balanced to room temperature, can not be heated to melt, the reagents that have been poured out should not be poured back into the bottle, to avoid contamination of the reagents in the bottle, reagent configuration or sample dilution, remember to mix well.
2. Each test should be done with a standard curve, and the user should estimate the amount of factor to be measured in the sample to decide whether to perform the appropriate dilution test on the sample so that the concentration of the protein of interest in the sample is within the optimal detection range of this kit.
3. When the tested sample needs to be diluted, if the standard/sample diluent is insufficient, PBST can be used instead. Please prepare PBST in advance.
4. Wash Liquid Configuration: Dilute the concentrated wash solution 1:30 with pure water (1 ml concentrated wash solution added to 29 ml of pure water). When the dilution and washing liquid is not enough, you can use 1 * PBST instead, if there is crystal precipitation in the washing liquid, please first warm to room temperature, gently mix well, until the crystal is completely dissolved and then prepared, the configuration process please use pure water, to avoid failure due to water pollution caused by experiments.
5. Antibody working solution configuration: 100ul biotin detection antibody (100 ×) Add to 10ml antibody diluent.
6. SABC working fluid configuration: 100ul SABC (100 ×) Add 10ml SABC diluent.
7. TMB Chromogenic Solution Configuration: Mix TMB Chromogenic Solution A and B 1:1 5 min before use, keep it away from light, and avoid bright light exposure during storage and color rendering.
8. Standard configuration: Take 7 1.5 ml centrifuge tubes, marked: S2, S3, S4, S5, S6, S7, blank, add universal dilution 200ul to each tube, remove the standard solution (S1) from the kit, aspirate 200ul with a pipette, move to the second tube S2, mix well on the mixer and pipette out 200ul to the third tube S3, so repeatedly double dilute to S7, the standard dilution is blank control hole brown, The standard product has a total of 8 holes, namely: S1, S2, S3, S4, S5, S6, S7, blank, used on the same day, the rest abandoned.




Inspection Process:

1. **Sample:** Add 50 μl of universal dilution to the blank wells, add 50 ul of the standard or sample to be measured in the remaining wells, seal the microplate plate with a sealing membrane, gently mix well and then place at 37 ° C, and incubate for 50 min.
2. **Wash:** Wash the microplate plate well 3 times with 1× wash solution, add 1× 300 μl of lotion per well, shake/soak for 1-2 minutes each time, and print dry onto the filter paper.
3. **Incubation of antibodies:** Add 100 ul of universal dilution to the blank wells, add 100 ul of the detection antibody working solution to the remaining wells, seal the microplate plate with a sealing membrane, gently mix well and then place at 37 ° C, and incubate for 50 min.
4. **Wash:** Ditto.
5. **Incubation of SABC**: add 100ul of SABC diluent to the blank hole, add 100ul of SABC working solution to the other holes, seal the enzyme label plate with a sealing membrane, gently mix it well, and then place it at 37 ℃ for incubation for 30 minutes.
6. **Wash:** Ditto.
7. **Color:** Add the pre-configured TMB mixture 100ul per well, seal the microplate plate with a sealing membrane, gently mix well and then place it at 37 ° C, react in the dark for 8-20 minutes, and the reaction result is blue.
8. **Termination reaction:**Add 50ul stop solution to each well, mix gently, at this time the blue turns to yellow, and measure the OD value at 450 nm with a microplate reader within 20 minutes.

Prompt:

- Dosing:Use disposable tips during experimental procedures to avoid cross-contamination. When adding, be careful not to have air bubbles, add the sample to the bottom of the microplate plate, try not to touch the well wall, and gently shake the mixture. When adding or adding reagents, if the time interval between the first well and the last well is too large, it will lead to different "incubation" times, which will significantly affect the accuracy and reproducibility of the measured values. Therefore, the one-time dosing time (including standards and all samples) is best controlled within 10 minutes.

- Incubation: In order to prevent sample evaporation, please seal the sealing membrane well during the experiment to avoid liquid evaporation, the next step should be carried out as soon as possible after washing the plate, and the microplate plate should be avoided in a dry state at any time, and the incubation time and temperature should be strictly observed.
- Washing: Adequate washing is very important, in each washing process, the washing liquid should be completely dried, the residual washing liquid in the reaction well during the washing process should be dried on the filter paper, do not put the filter paper directly into the reaction well to absorb water, and at the same time eliminate the residual liquid and finger prints at the bottom of the plate to avoid affecting the final microplate reader reading.
- Color development time control: The color rendering time in the instructions is for reference, due to the difference in user laboratory conditions, the best color development time will vary, after adding the substrate, please observe the color change of the reaction well regularly (for example, observe every 5 minutes), if the color is dark, please terminate the reaction in advance. The reaction can be terminated when the standard curve has a significant gradient and the S7 well is visible to the naked eye in a faint blue color, avoiding too strong a reaction that affects the microplate reader optical density reading.
- This kit uses acid as a termination solution, which is corrosive and should be used without contact with clothing or exposed skin such as eyes and hands.

## Result judgment and calculation:

1. The blank wells are set as control wells, and after subtracting the absorbance values of all the standards and samples from the blank wells, the resulting data can be directly plotted on the coordinate paper, such as when the absorbance value (OD) value of the blank well is lower than 0.1, it can also be directly calculated.
2. Take the standard concentration as the abscissa, the OD value as the ordinate, draw the standard curve by hand or with software, calculate the corresponding content according to the sample OD value, and then multiply it by the dilution multiple.
3. If the S1 detection OD value exceeds the detection range of the microplate reader, its value can be discarded for statistical analysis without affecting the experimental results.

## Recovery:

Known proteins were added to serum and plasma samples, the assay was repeated and their means were calculated, and the recovery rate was the ratio of the measured value to the theoretical value, and the test was within the recovery range.

| Sample | Recovery rate range(%) |
|---|---|
| Serum | 88-96 |
| EDTA anticoagulant plasma | 87-96 |
| Heparin sodium anticoagulant plasma | 83-95 |

Linear range:

A certain amount of protein of interest is added to the serum and plasma samples, and the sample to be measured is double-diluted, and the linear range is the ratio of the measured value of the protein content of the target protein in the diluted sample to the theoretical value.

Precision:

Precision is expressed in the coefficient of variation CV of the sample measurement value. CV(%) = SD/mean×100

The SD value is the standard deviation, which is the arithmetic square root of the arithmetic mean of the squared deviation from the mean difference, expressed in σ, and the standard deviation is also known as the standard deviation, or experimental standard deviation.

Intra-batch difference: Take the same batch of kits to quantitatively detect low, medium and high value fixed value samples, and each sample is continuously determined 20 times, and the average and SD values of samples of different concentrations are calculated respectively. Intra-batch difference: CV<9%。

Batch-to-batch difference: 3 different batches of kits were selected for quantitative determination of low, medium and high value fixed value samples, and each sample was repeated 8 times using the same kit, and the average and SD values of samples of different concentrations were calculated, respectively. Batch-to-batch difference: CV<10% 。

Stability:

The kit was measured to be stored at the recommended temperature during the validity period and had a decrease in activity of less than 5%. In order to reduce the influence of external factors on the detection value before and after the destruction of the kit, the environmental conditions of the laboratory should be as

consistent as possible, especially the temperature and incubation conditions in the laboratory, and then the same experimenter can reduce human error.

Problem analysis:

If the experimental results are not ideal, please take photos of the color rendering results in time, save the experimental data, retain the slats used and unused reagents, fill in the after-sales service form (download and fill in online), and then contact our technical support to solve the problem for you, and you can also refer to the following information:

Standard deviation:

| Possible causes | Countermeasures accordingly |
|---|---|
| The standard solution is misconfigured | Dosing or dosing is not permitted, check the pipette and tip to confirm that the correct dilution is being made. |
| Improper re-dissolution of the standard | Centrifuge before opening the cap to check for insoluble matter after resolution. |
| The standard has degraded | Preserve and dispose of standards in the recommended manner. |
| Incomplete washing | Ensure washing time and number of washes and dosing per well. |
| The scale of the curve is not suitable | Try drawing curves with different scales. |
| Pipette dosing error | Use calibrated pipetting correctly. |

No signal

| Possible causes | Countermeasures accordingly |
|---|---|
| The target content is below the detection range | Reduce the dilution multiple of the sample or concentrate the sample. |
| Sample type is not applicable | For sample types that have not been validated, the detection signal may be weakened or the validated sample type may not be used as a positive control for simultaneous testing. |
| Detect the compatibility of the buffer | Ensure that the detection buffer is compatible with the target |

| | |
|---|---|
| Incorrect sample preparation | Make sure to perform the correct sample preparation/dilution. Samples may not be compatible with the microtiter plate assay form. |
| Insufficient antibodies | Try different antibody concentrations/dilutions. |
| The incubation temperature is too low | Should be at room temperature, or the temperature recommended by the reagent protocol. |
| The wavelength is incorrect | Confirm the wavelength and read the plate again. |
| The well plates are washed vigorously | Check and make sure the automatic washing system is at the correct pressure. If washing manually, gently aspirate the rinse buffer. |
| The holes dry out | Do not allow the holes to dry out after the assay has begun. All incubation steps are sealed with parafilm or tape to seal the well plate. |
| The color development speed of the enzyme reaction is slow | Prepare the substrate solution before use. Make sure that the mother liquor has not expired or been contaminated. Extend the incubation time. |
| The kit is not sufficiently balanced | Reagents are balanced at room temperature for at least 20 minutes, ensuring that all reagents are balanced to room temperature. |

The coefficient of variation is large

| Possible causes | Countermeasures accordingly |
|---|---|
| There are bubbles in the wells | Before reading the plate, make sure there are no air bubbles. |
| Uneven/inadequate well wash | Check that all nozzles of the plate washer are unobstructed. Wash using the recommended method. |
| Reagents are not mixed well | Make sure all reagents are mixed well. |
| Edge effects | Make sure the well plate and all reagents are at room temperature. |
| Inconsistent sample preparation or storage conditions | Ensure consistent sample preparation and use optimal sample preservation conditions (e.g., minimize repeated freeze-thaw). |

High background:

| Possible causes | Countermeasures accordingly |
|---|---|

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|---|---|
| Insufficient well washing | Wash as recommended by the protocol. |
| Wash buffer contamination | Prepare fresh wash buffer. |
| Too many detection reagents | Ensure that the reagent is properly diluted or reduce the recommended concentration of the reagent for detection. |
| Blocking buffer is ineffective | Try different blocking agents and/or adding blocking agents to the wash buffer. |
| Salt concentration of incubation/wash buffer | Increasing salt concentrations may reduce nonspecificity and/or weaken off-target interactions. |
| The time after adding the stop solution before reading the plate is too long | Read the plate immediately after adding the stop solution. |
| High antibody concentration | Try different dilutions to get the best results. |
| Substrate incubation is performed under light | Substrate incubation should be carried out protected from light |
| Sediment is generated in the wells after the substrate is added | Increase the dilution multiple of the sample or reduce the substrate concentration. |
| The well plate is dirty | Clean the bottom of the well plate. |
| The chromogenic solution deteriorates or the reagent expires | Check the validity period of the kit and use it within the validity period |
| Changes in incubation time and temperature | Follow the recommended time and temperature on the instructions |
| The sealing film is reused | Replace the used sealing film in time |

Sensitivity is low

| Possible causes | Countermeasures accordingly |
|---|---|
| ELISA kits are not properly stored | Save all reagents as recommended. |
| Insufficient targets | Concentrate the sample or reduce the sample dilution. |
| Detection reagent inactivation | Make sure the reporting enzyme/fluorescein has the expected activity. |

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|---|---|
| The microplate reader settings are incorrect | During the assay, ensure that the microplate reader is set to the correct absorption wavelength or excitation/emission wavelength. |
| Microtiter plates are not effective in adsorbing targets | Covalently bind the target to a microtiter plate. |
| Incompatible sample types (e.g., serum versus cell extracts) | For sample species that have not been validated, the detection signal may be weakened or absent. Simultaneous testing using a validated sample type as a positive control. |
| Buffer or sample composition interference | Confirm the presence of interfering compounds in the reagent, for example, sodium azide in an antibody inhibits the HRPase. |
| Mix reagents from different kits | Avoid mixing reagents from different kits. |
| The kit is not sufficiently balanced | Reagents are balanced at room temperature for at least 20 minutes, ensuring that all reagents are balanced to room temperature. |

Statement:

1. The company is only responsible for the kit itself, and is not responsible for the sample consumption caused by the use of the kit, and the user fully considers the possible amount of the sample before use, and reserves sufficient samples. The final experimental results are closely related to the effectiveness of the reagents, the relevant operations of the experimenter, and the experimental environment.
2. Due to the existing conditions and scientific and technological level can not be the supplier to provide all the raw materials for comprehensive identification and analysis, this product may have a certain quality and technical risks, such as experimental failure, the user himself bears the risk, the company does not bear any experimental failure losses other than the kit.
3. If the sample examined is not included in the sample listed in the specification, it is recommended to perform a pre-experiment to verify its validity and pay attention to the retention of the sample.
4. Tissue homogenates or cell extracts prepared using chemical lysates may bias ELISA experimental results due to the introduction of certain chemicals.
5. Cell culture supernatant samples, because of the many interfering factors of this type of sample, including cell status, cell viability, cell number, and sampling time, there may be undetectable conditions.
6. Some natural or recombinant proteins, including prokaryotic and eukaryotic recombinant proteins, may not be detected because they do not match the detection and capture antibodies used in this product.
7. Different batches of the same product may have a little difference, such as: detection limits, sensitivity and color development time, etc., please refer to the instructions in the kit, the website electronic version of the manual for reference only.

8. Only by using all the reagents in the kit can the detection effect be guaranteed, and the products of other manufacturers cannot be mixed, and the best test results will be obtained only by strictly abiding by the experimental instructions of this kit.
9. Avoid exposing reagents to bright light during storage and transportation, and all reagent bottle caps must be tightly closed to prevent evaporation and microbial contamination, resulting in reagent failure or contamination with inaccurate results.
10. There may be a little water-like substance in the wells of the newly opened microplate plate, which is normal and will not have any impact on the results of the experiment, and the microplate plate should be removed from the bag when it is used, please do not remove it in advance.
11. Changes in sample preparation and during each procedure can lead to different experimental results, so in order to improve the reproducibility of experimental results, each step of the experiment needs to be strictly controlled.
12. Kits undergo strict quality inspection before leaving the factory, but due to the differences in transportation conditions and laboratory conditions, it may cause inconsistencies between the experimental results and the factory results or the increase in the difference between batches of kits.
13. This kit is not compared with other manufacturers' similar kits or products with different methods to detect the same protein of interest, so inconsistencies in the test results are not excluded.
14. The immunogen used to prepare the antibody in the kit is usually a recombinant protein, but because the fragments selected for the preparation of the recombinant protein, the expression system, the purification method, etc. are different, we cannot guarantee that the kit can be used for the detection of recombinant proteins of other companies.
15. The kit may not be suitable for the detection of special experimental samples whose effectiveness of the experiment itself is uncertain, such as samples such as gene knockout experiments.
16. The kit is for research purposes only and if used for clinical diagnostics or any other purpose, we will not be responsible for or liable for any problems arising therefrom.