（本试剂盒仅作体外科研使用，不可用于临床诊断。）

Rat Aggrecan Elisa Kit-Elisa 试剂盒

货号：U96-1717E

产品保质期及产品批号见试剂盒外包装标签。

使用前请仔细阅读此说明书，如果有任何问题请及时联系我们！

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产品名称：Rat Aggrecan Elisa Kit-Elisa 试剂盒

产品货号：U96-1717E

规格：48T/96T

检测范围：0.312 - 20ng/ml

灵敏度：＜0.06ng/ml

精密度：板内变异系数均＜9%，板间变异系数均＜10%。

保存温度：4℃

有效期：6 个月

标准曲线对应浓度（ng/ml）：

S1	S2	S3	S4	S5	S6	S7	Blank
20.0	10.0	5.0	2.5	1.25	0.62	0.312	0

检测原理：

本试剂盒采用双抗体夹心 ELISA 法。试剂盒提供的酶标板已包被捕获抗体，实验时将待检样品（或标准品）加入酶标板反应孔中，捕获抗体结合目的蛋白。加入生物素标记检测抗体与目的蛋白结合，再加入 SABC 与标记抗体结合，形成捕获抗体-目的蛋白-检测抗体-SABC 免疫复合物，游离成分均被洗去。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 450nm 波长处检测吸光度(OD)，目的蛋白浓度与 OD450 值之间呈正相关，通过绘制标准曲线计算出样品中目的蛋白的浓度。

试剂盒组分（4℃保存）：

内容物	规格	
	48T	96T
酶标板	8 孔×6 条	8 孔×12 条
标准品(S)	1 支	1 支
标准品/样本稀释液	12ml	12ml
生物素标记检测抗体(100×)	60 μl	120μl
生物素标记检测抗体稀释液	6ml	12ml
SABC(100×)	60μl	120μl
SABC 稀释液	6ml	12ml
TMB 显色液(A/B)	6ml	12ml
终止液	3ml	6ml
30×洗涤缓冲液	30ml	30ml
封板膜	2 张	4 张
产品说明书	1 份	1 份

注意：使用前请检查试剂盒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。

保存温度及有效期：

1、未拆封的试剂盒 4℃保存，6 个月内有效。

2、拆封后的试剂盒，将未使用的酶标条用密封袋装好，4℃保存，1 个月内有效。

需要自备的物品：

1、酶标仪（含有 450nm 滤光片），使用前提前预热酶标仪。

2、系列高精度移液器及一次性吸头，检测样品较多时，建议使用多通道移液器。

3、37℃恒温箱。

4、干净的 1.5ml 离心管。

5、蒸馏水或去离子水。

6、吸水纸。

样品的收集和保存：

1、血清：全血样品于室温放置 2 小时或 4℃过夜，然后 4℃，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。

2、血浆：用 EDTA 或肝素钠抗凝管采集全血样品，采集后 30 分钟内置于 4℃冰箱，1,000×g 离心 15 分钟，取上清即可检测。或取上清置于≤ -20℃冷冻保存，避免反复冻融。

3、组织匀浆：用预冷的 PBS (0.01mol/L， pH=7.4) 清洗组织，去除残留血液，冰上切割组织后称重。按组织重量(g): PBS 体积(ml) =1:9 的比例，加入 9 倍体积的 PBS（比如 1g 的组织样品加入 9ml 的 PBS，具体体积可根据实验需要适当调整，并做好记录），加入玻璃匀浆器中，在冰上充分研磨。可以对匀浆液进行反复冻融或超声破碎以充分匀浆。最后将匀浆液于 4℃，3000×g 离心 15 分钟，取上清即可检测（如需要可取部分上清进行 BCA 蛋白定量）。或取上清置于≤ -20℃冷冻保存，避免反复冻融。

4、细胞裂解液：贴壁细胞用预冷的 PBS 轻轻清洗，胰蛋白酶消化，1,000×g 离心 5 分钟后收集细胞；悬浮细胞可直接离心收集。收集到的细胞用预冷的 PBS 洗涤 3 次。PBS 稀释细胞悬液，细胞浓度达到 10^6 个/ml 左右。通过反复冻融或超声破碎使细胞裂解。4℃，3,000×g 离心 15 分钟，取上清即可检测。或取上清置于≤ -20℃冷冻保存，避免反复冻融。

5、细胞培养上清或其他生物体液：收集液体后于 4℃，3000×g 离心 15 分钟，取上清即可检测。

样本处理相关试剂推荐：PBS 缓冲液（货号：U21-259B），0.25%胰蛋白酶-EDTA 消化液（货号：U31-323C），0.25%胰蛋白酶消化液（货号：U31-324C）。

注意事项：

1、本试剂盒仅供体外科研使用，不可用于临床诊断。

2、实验员请穿着实验服并佩戴一次性乳胶手套做好防护工作。特别是检测血液或者其他体液样品时，请严格按照国家生物试验室安全防护条例执行。

3、刚开启的酶标板孔中可能会有少许液体，此为正常现象，不会对实验结果造成任何影响。拆封后未使用的酶标条用密封袋装好，4℃条件下可保存一个月。

4、请勿重复使用已稀释过的标准品、生物素标记检测抗体、SABC。

5、酶标仪需要安装检测 450nm 波长的滤光片，建议使用酶标仪前 15 分钟预热。

6、请勿使用其他批号或其他来源的试剂混合或替代本试剂盒中的试剂。

7、试验中所用的离心管和吸头均为一次性使用，严禁混用。

8、请勿使用过期的试剂。

9、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。

10、样品收集后若在 1 周内进行检测可保存于 4℃，若不能及时检测，应按一次使用量分装，冻存于-20℃（1 个月内检测），或-80℃（3 个月内检测），避免反复冻融。在检测前，冷冻的样本应在室温缓慢融化后离心去除冻融过程产生的沉淀物。室温轻柔混匀后使用。

11、本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。

12、若所检样本不在说明书所列样本之中，建议做预实验验证其检测有效性。

13、若使用化学裂解液制备组织匀浆或细胞裂解液，由于引入某些化学物质可能会导致 ELISA 检测结果出现偏差。

14、某些重组蛋白可能与试剂盒中捕获或检测抗体不匹配而出现不能检测的情况。

15、使用前 TMB 显色液应为无色透明溶液，如果发现颜色异常，请及时与我们联系。

16、初次使用试剂盒时，应将各种试剂管离心数分钟，以便试剂集中到管底。

17、实验过程中要严格避免酶标板干燥。

18、覆盖和揭开封板膜时应小心操作，避免液体溅出。

样本稀释原则：

请提前预估样品中目的蛋白的含量，以此决定是否对样品进行适当稀释检测，以便使样品中目的蛋白浓度处于本试剂盒的最佳检测范围内。

参考稀释方案如下：

1、代测样品目的蛋白含量超低：浓缩后检测。

2、代测样品目的蛋白含量低：直接原液检测。

3、代测样品目的蛋白含量中：稀释后检测。一般按 1:10 稀释，270μl 稀释液加 30μl 样品。

4、代测样品目的蛋白含量高：稀释后检测。一般按 1:100 稀释，297μl 稀释液加 3μl 样品。

5、代测样品目的蛋白含量超高：稀释后检测。一般按 1:1,000-1:10,000。

样品 1000 倍稀释：分两步稀释。取 5μl 样品转移至 95μl 稀释液内，记作 A 液，此为 20 倍稀释；再取 A 液 5μl 转移至 245μl 稀释液内，此为 50 倍稀释，总共稀释 1,000 倍。

样品 10,000 倍稀释：分三步稀释。取 5μl 样品转移至 195μl 稀释液内，记作 A 液，此为 40 倍稀释；再取 A 液 5μl 转移至 245μl 稀释液内，记作 B 液，此为 50 倍稀释；最后取 B 液 60μl 转移至 240μl 稀释液内，此为 5 倍稀释，总共稀释 1,000 倍。

每步稀释时取液量不少于 3μl，稀释倍数不超过 100 倍。每步稀释都需混合均匀，避免产生气泡。

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），以上方案仅作参考，最好做预实验以确定稀释倍数，并详细记录。

洗板方法：

手工洗板：

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，酶标板朝下用力拍打几次，每孔加入 1×洗涤缓冲液 300μl，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，应注意观察每个反应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内放置的时间。

检测前准备工作：

1、实验前 30 分钟将所有试剂和样品平衡至室温（不能加热使其融解）。如果试剂盒需分多次使用，请仅取出本次实验所需的酶标条和试剂，剩余酶标条和试剂需按照要求保存好。已经倒出的试剂请勿倒回瓶中，避免瓶中试剂污染，试剂配制或样品稀释时，切记要混合均匀。

2、每次检测都应该做标准曲线，实验员应该预估样品中目的蛋白的含量，以此决定是否对样品进行适当稀释检测，以便使样品中目的蛋白浓度处于本试剂盒的最佳检测范围内。

3、当样品需要稀释时，如果标准品/样品稀释液不够用，可以用 1×PBST 替代，请提前准备好 1×PBST。

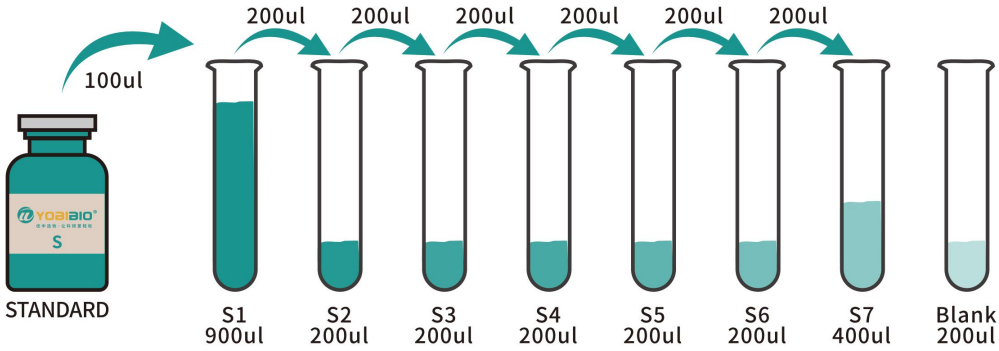
4、配制洗涤液 (1×)：用蒸馏水或者去离子水 1:30 稀释 30×缓冲洗涤液（1ml 缓冲洗涤液加入 29ml 的蒸馏水或去离子水），当稀释液或洗涤液不够用，可以用 1×PBST 替代。提示：从冰箱中取出的 30×缓冲洗涤液可能有结晶析出，属于正常现象，请先温育至室温，轻轻混匀至结晶完全溶解再进行洗涤液配制操作。配置好的洗涤液 (1×) 仅作一次性使用，当日使用完毕。

5、配制检测抗体工作液：实验前计算当次实验所需用量（以 100μl/孔计算），实际配制时应多配制 100-200μl。使用前 15 分钟，将生物素标记检测抗体 (100×) 于 800×g 离心 1 分钟，用生物素标记检测抗体稀释液将生物素标记检测抗体 (100×) 稀释成 1×检测抗体工作液（比如：10μl 100×检测抗体 +990μl 抗体稀释液）。现配现用。

6、配制 SABC 工作液：实验前计算当次实验所需用量（以 100μl/孔计算），实际配制时应多配制 100-200μl。使用前 15 分钟，将 SABC 于 800×g 离心 1 分钟，用 SABC 稀释液将 SABC (100×) 稀释成 1×SABC 工作液（比如：10μl 100×SABC+990μl SABC 稀释液）。现配现用。

7、配制 TMB 显色液：实验前计算当次实验所需用量（以 100μl/孔计算），实际配制时应多配制 100-200μl。使用前 5 分钟，将 TMB 显色液 A 液和 B 液 1:1 混合均匀，避光放置备用，在储存和显色时均避免强光照射。

标准品工作液：取 8 个 1.5ml 离心管，分别标记 S1、S2、S3、S4、S5、S6、S7、Blank。第一管 S1 中加入标准品/样品稀释液 900μl，第二至第八管中分别加入标准品/样品稀释液 200μl。在第一管 S1 中加入标准品 (S) 溶液 100μl，置于漩涡混合器上混匀后吸出 200μl，移至第二管，如此反复作对倍稀释至第七管 (S7)，第八管为空白对照。（标准品的用量及标准曲线范围也可根据自己需要配制）



提示：

1、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。

2、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。

3、洗涤:充分的洗涤非常重要，在每次洗过程中，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。

4、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。

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 50μl 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，每孔加入洗涤液 300μl，每次浸泡/震荡 1-2 分钟，

倒掉或者吸去后在吸水纸上拍干。
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3、孵育抗体：空白孔中加入 100μl 的抗体稀释液，其余孔中各加入检测抗体工作液 100μl，将酶标板用封板膜封好，轻轻混匀后静置于 37℃，孵育 50 分钟。

4、洗板：同步骤 2。

5、孵育 SABC：空白孔中加入 100μl 的 SABC 稀释液，其余孔中各加入 SABC 工作液 100μl，将酶标板用封板膜封好，轻轻混匀后静置于 37℃，孵育 30 分钟。

6、洗板：同步骤 2。

7、显色：每孔加入提前配制好的 TMB 混合液 100μl，将酶标板用封板膜封好，轻轻混匀后静置于 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 50μl 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 处检测 OD 值。

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 OD 值减去空白孔的 OD 值后，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。

样本类型	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。

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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。

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。

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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。

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
标准品溶液配制有误	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	开盖前先离心，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器。

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	使用前应处于室温，或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。确保母液未过期或污染。延长孵育时间。
试剂盒没有充分平衡	试剂盒室温平衡至少 20 分钟，确保所有试剂已平衡至室温。

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/洗涤缓冲液盐浓度改变	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	加入终止液后立即读板。
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。
加入底物后有沉淀生成	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。
样品类型不兼容质（例如血清与细胞提取物）	对于未来验证过的样品种属，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
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。

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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）。

(This kit is for research use only and is not intended for clinical diagnosis.)

Rat Aggrecan Elisa Kit-Elisa 试剂盒

Product Code: U96-1717E

Product shelf life and batch number are shown on the outer packaging label.

Please read this manual carefully before use. If you have any questions, please contact us promptly!

Phone: 400-681-8582

QQ: 1296725867

Email: service@scigebio.com

Website: www.yobibio.com

Product Name: Rat Aggrecan Elisa Kit-Elisa 试剂盒

Product Code: U96-1717E

Specification: 48T/96T

Detection Range: 0.312 - 20ng/ml

Sensitivity: ＜0.06ng/ml

Precision: Intra-assay coefficient of variation (CV) < 9%; Inter-assay CV < 10%.

Storage Temperature: 4℃

Shelf Life: 6 months

Standard Curve Reference Concentrations（ng/ml）：

S1	S2	S3	S4	S5	S6	S7	Blank
20.0	10.0	5.0	2.5	1.25	0.62	0.312	0

Detection Principle:

This kit uses a sandwich ELISA method. The microplate provided is pre-coated with capture antibodies. During testing, add test samples (or standards) to the reaction wells. The capture antibodies bind the target protein. Then add biotin-labeled detection antibodies to bind the target protein, followed by SABC binding to the detection antibodies, forming a capture antibody-target protein-detection antibody-SABC immune complex. Wash away unbound components. Add chromogenic substrate (TMB); TMB turns blue catalyzed by horseradish peroxidase. After adding stop solution, it turns yellow. Measure OD at 450nm using a microplate reader. The target protein concentration positively correlates with OD450 values. Calculate sample concentration via a standard curve.

Kit Components (Store at 4℃):

Component	Specifications	
	48T	96T
Pre-coated Microplate	8 wells×6 strips	8 wells×12 strips
Standard (S)	1 vial	1 vial
Standard/Sample Diluent	6ml	12ml
Biotin-Labeled Detection Antibody (100×)	60μl	120μl
Detection Antibody Diluent	6ml	12ml
SABC (100×)	60μl	120μl
SABC Diluent	6ml	12ml
TMB Substrate (A/B)	6ml	12ml
Stop Solution	3ml	6ml
30× Wash Buffer Concentrate	30ml	30ml
Adhesive Sealing Film	2sheets	4 sheets
Instruction Manual	1 copy	1 copy

Note: Before use, check that the kit labels and quantities match the packing list. Tighten all reagent bottle caps to prevent evaporation and microbial contamination. Reagent volumes are subject to the actual shipped version of the manual. Relevant reagents are filled slightly above the labeled volume during aliquoting; always measure for use rather than pour directly.

Storage Conditions and Validity Period:

Unopened kits are valid for 6 months when stored at 4℃.

For opened kits, unused microplate strips must be sealed in airtight bags and stored at 4℃; these remain valid for 1 month.

User-Supplied Equipment Required:

1.Microplate reader equipped with 450nm filter (pre-warm before use)

2.High-precision pipettes and disposable tips (multichannel pipettes recommended for high-throughput

3. 37℃ constant-temperature incubator

4. Clean 1.5ml microcentrifuge tubes

5. Distilled or deionized water

6. Absorbent paper

Sample Collection and Preservation:

1.Serum: For whole blood samples, place at room temperature for 2 hours or at 4℃ overnight. Then centrifuge at 4℃, 1,000×g for 15min. Collect the supernatant for immediate testing. Alternatively, store the supernatant at ≤ -20℃; avoid repeated freeze-thaw cycles.

2.Plasma: Collect whole blood samples in EDTA or heparin sodium anticoagulant tubes. Within 30 minutes post-collection, place samples at 4℃, centrifuge at 1,000×g for 15 minutes, then collect the supernatant for immediate testing. Alternatively, store the supernatant at ≤ -20℃; avoid repeated freeze-thaw cycles.

3.Tissue Homogenization: Rinse tissues with pre-cooled PBS (0.01mol/L, pH=7.4) to remove residual blood. Weigh tissues after dissection on ice. Add PBS at a tissue(g):PBS(ml) ratio of 1:9 (e.g., 9ml PBS per 1g tissue; adjust volume as needed and record). Transfer to a glass homogenizer and grind thoroughly on ice. Perform repeated freeze-thaw cycles or ultrasonic disruption for complete homogenization. Centrifuge the homogenate at 4°C, 3,000×g for 15min. Collect supernatant for immediate testing (optionally quantify protein via BCA assay). Alternatively, store supernatant at ≤ -20°C; avoid repeated freeze-thaw cycles.

4.Cell Lysate: Adherent cells gently clean with pre-cooled PBS, trypsin digest, centrifuge 1,000×g 5 min collect cells; suspension cells directly centrifuge collect. Collected cells wash 3 times with pre-cooled PBS. PBS dilute cell suspension, cell concentration reach ~10^6 cellls/mL. Repeated freeze-thaw or ultrasonic disruption lyse cells. 4℃, 3,000×g centrifuge 15min, collect supernatant test. Or store supernatant at ≤ -20℃ freeze, avoid repeated freeze-thaw.

5.Cell Culture Supernatant/Biofluids: Centrifuge samples at 3,000×g for 15min at 4℃. Test supernatant immediately.

Recommended Reagents for Sample Processing: PBS Buffer (Cat# U21-259B); 0.25% Trypsin-EDTA Solution (Cat# U31-323C); 0.25% Trypsin Solution (Cat# U31-324C)

Precautions for Using the Reagent Kit：

1. This kit for in vitro research use only, not for clinical diagnosis.

2. Operators wear lab coats and disposable latex gloves. Especially when testing blood or bodily fluids, strictly follow national biosafety lab protection regulations.

3. Newly opened microplate wells may have slight liquid, normal phenomenon no impact on results. Unused strips after opening seal in bags, store at 4℃≤1 month.

4. Do not reuse diluted standards, biotin-labeled detection antibodies, or SABC.

5. Microplate reader requires 450nm filter. Preheat 15 minutes before use.

6. Do not mix or substitute reagents from other batches/sources.

7. Centrifuge tubes and pipette tips single-use only, strictly prohibit cross-use.

8. Do not use expired reagents.

9. Blood collection tubes must be disposable endotoxin-free. Avoid hemolyzed or lipemic samples.

10. If samples will be tested within one week after collection, store at 4℃; if cannot be tested promptly, aliquot by single-use quantity, store frozen at -20℃ (detect within one month) or -80℃ (detect within three months), avoid repeated freeze-thaw. Before detection, thaw frozen samples slowly at room temperature, centrifuge to remove precipitates generated during freeze-thaw, gently mix at room temperature then use.

11. The detection range of this kit is not equivalent to the concentration range of target analyte in samples. If the concentration of target analyte in samples is too high or too low, conduct appropriate samples dilution or concentration.

12. For samples not listed in manual, conduct pre-experiment validation.

13. Tissue/cell lysates prepared with chemical lysates may cause ELISA deviations.

14.Some recombinant proteins may be undetectable due to antibody mismatch.

15. TMB substrate should be colorless clear solution before use. Contact us immediately if color abnormal.

16. During first kit use: Centrifuge all reagent tubes several minutes to concentrate liquid.

17. Strictly avoid microplate drying during experiments.

18. Handle sealing/unsealing of plate cover carefully to prevent splashing.

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Principles for Sample Dilution：

Estimate the target protein concentration in samples rior to testing. Dilute samples appropriately to ensure the target protein concentration falls within the optimal detection range of the reagent kit.

Reference Dilution Schemes:

In advance estimate the content of target protein in samples to determine whether to conduct appropriate sample dilution testing, in order to make the target protein concentration in samples be within the best detection range of this kit.

Reference dilution schemes as below:

Target protein content ultra-low in test sample: Concentrate then test.

Target protein content low in test sample: Test neat solution directly.

Target protein content medium in test sample: Dilute then test. Generally dilute 1:10 (e.g., 270μl diluent + 30μl sample).

Target protein content high in test sample: Dilute then test. Generally dilute 1:100 (e.g., 297μl diluent + 3μl sample).

Target protein content ultra-high in test sample: Dilute then test. Generally dilute 1:1,000-1:10,000.

Sample 1000× dilution: Dilute in two steps. Transfer 5μl sample to 95μl diluent, record as Solution A (20× dilution); then transfer 5μl Solution A to 245μl diluent (50× dilution), total dilution 1,000 times.

Sample 10,000× dilution: Dilute in three steps. Transfer 5μl sample to 195μl diluent, record as Solution A (40× dilution); then transfer 5μl Solution A to 245μl diluent, record as Solution B (50× dilution); finally transfer 60μl Solution B to 240μl diluent (5× dilution), total dilution 1,000 times.

Each dilution step: Sample volume ≥ 3μl, dilution factor ≤ 100×. Mix thoroughly after each step, avoid bubbles.

For serum, plasma, lavage fluid, urine, pleural effusion, saliva samples: Test neat (except individual indicators). Above schemes for reference only, best do pre-experiment to confirm dilution factor, record details.

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Plate Washing Method:

Manual Plate Washing:

Remove or discard the liquid from the microplate wells without touching the walls, layer several sheets of absorbent paper on the lab bench, turn the microplate upside down and tap firmly several times, then add 300µl of 1× washing buffer to each well, soak for 1-2 minutes, and repeat this process several times.

Automated Plate Washing:

1.Before washing, check if the wash buffer bottles and distilled water bottles are sufficiently filled, and whether the waste bottle is full.

2.During the self-test, observe if wash buffer flows smoothly through the channels and drains properly.

3.During washing, monitor whether each well is fully filled without overflow, ensure complete liquid aspiration from all wells, and strictly maintain the required incubation time for the wash buffer in the wells.

Pre-Test Preparations:

1.Balance all reagents and samples to room temperature 30 minutes before the experiment (do not heat to dissolve). If the kit will be used multiple times, only remove the microplate strips and reagents needed for the current experiment. Store remaining strips and reagents as required. Do not pour reagents back into bottles to avoid contamination. Mix reagents/samples thoroughly during preparation or dilution.

1.Generate a new standard curve for each test. Estimate target protein concentration in samples to determine if dilution is needed, ensuring values fall within the kit's optimal detection range.

2.If diluent for standards/samples is insufficient, substitute with 1× PBST (prepare in advance).

3.Prepare 1× wash buffer: Dilute 30× concentrated wash buffer 1:30 with distilled/deionized water (1ml concentrate + 29ml water). If 1× buffer is insufficient, use 1× PBST instead. Note: Crystallization in

refrigerated 30× buffer is normal – incubate to room temperature and mix gently until dissolved before dilution. Prepared 1× wash buffer is single-use only; discard unused portion same day.

4.Detection antibody working solution: Calculate required volume (based on 100µl/well), plus 100-200µl excess. Centrifuge biotin-labeled detection antibody (100×) at 800×g for 1 min, then dilute with antibody diluent to 1× working solution (e.g., 10µl 100× antibody + 990µl diluent). Prepare immediately before use.

5.SABC working solution: Calculate required volume (100µl/well), plus 100-200µl excess. Centrifuge

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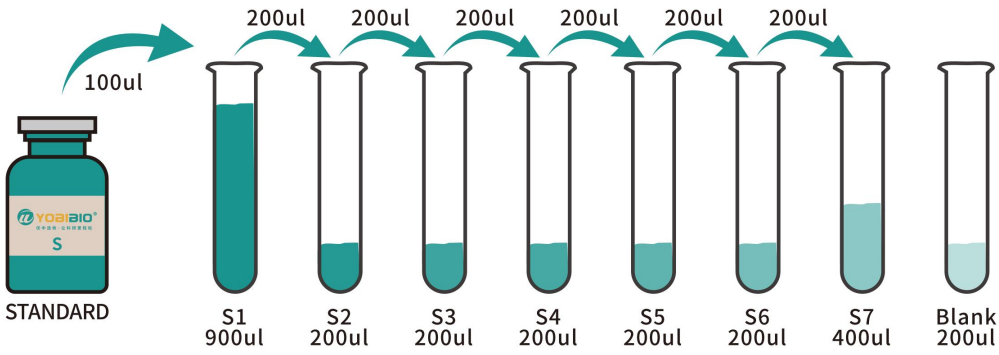
SABC (100×) at 800×g for 1 min, then dilute with SABC diluent to 1× working solution (e.g., 10µl 100× SABC + 990µl diluent). Prepare immediately before use.

6.TMB substrate solution: Calculate required volume (100µl/well), plus 100–200µl excess. Mix TMB Solution A and B 1:1, 5 min before use. Protect from light during storage and incubation.

7.Standard working solution: Label eight 1.5ml tubes: S1, S2, S3, S4, S5, S6, S7, Blank. Add 900µl diluent to S1 and 200µl to others. Add 100µl standard (S) to S1, vortex, transfer 200µl to S2. Repeat serial dilution to S7. Blank is diluent only. Adjust standard range as needed.



Critical Notes:

1.Loading: Use disposable tips to avoid cross-contamination. Load samples to the bottom of wells without bubbles or touching walls. Gently mix. Complete all loading (standards + samples) within 10 min to ensure consistent incubation time.

2.Incubation: Seal wells with plate sealer to prevent evaporation. Avoid plate drying at all times. Strictly adhere to incubation time/temperature.

3.Washing: Remove all residual liquid after each wash. Tap plate firmly on absorbent paper to dry – do not insert paper into wells. Wipe plate bottom to remove liquid/fingerprints.

4.Color development: Incubation time in the manual is a reference only. Monitor color change every 5 min after adding substrate. Stop reaction early if color becomes too intense. Terminate when the standard curve shows clear gradients and faint blue is visible in S7.

Stop solution contains corrosive acid. Avoid contact with skin, eyes, or clothing.

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Detection Procedure:

1.Sample Loading:Blank well: Add 50µl standard/sample diluent.Other wells: Add 50µl standards or test samples.Seal plate and incubate at 37℃ for 50min with gentle shaking.

2.Washing: Wash plate 3 times with 300µl/well of 1× wash buffer, soaking/shaking for 1-2 min per cycle. Aspirate liquid and tap plate dry on absorbent paper.

3.Antibody Incubation:Blank well: Add 100µl antibody diluent. Other wells: Add 100µl detection antibody working solution. Seal plate and incubate at 37℃ for 50 min without shaking.

4.Washing: Repeat Step 2.

5.SABC Incubation:Blank well: Add 100µl SABC diluent. Other wells: Add 100µl SABC working solution.Seal plate and incubate at 37℃ for 30 min without shaking.

6.Washing: Repeat Step 2.

7.Color Development: Add 100µl/well of pre-mixed TMB substrate, seal plate, and incubate at 37℃ in darkness for 8-20 min until blue color develops.

8.Reaction Termination: Add 50µl stop solution per well. Mix gently until blue turns yellow. Read OD at 450nm within 20 min.

Result Calculation:

1.Blank well set as control well, all standards and samples OD values subtract blank well OD value, obtained data directly plot curve on coordinate paper, if blank well absorption value (OD) value below 0.1, can also directly calculate.

2.Use standard concentration as horizontal axis, OD value as vertical axis, plot standard curve by manual or software. Based on sample OD value, calculate corresponding content then multiply dilution factor.

3.If S1 test OD value exceeds the microplate reader detection range, can discard its value conduct statistical analysis, not affect experimental results.

Recovery Rate:

Separately in serum and plasma samples add known protein, repeat tests and calculate their mean, recovery rate is measured value divided by theoretical value, through testing all within recovery range.

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Sample Type	Recovery Range(%)
Serum	88-96
EDTA plasma	87-96
Heparin plasma	83-95

Linearity Range:

In serum and plasma samples add a certain amount target protein, and serially dilute test samples, linear range is diluted samples target protein content measured value divided by theoretical value ratio.

Precision:

Precision expressed as coefficient of variation (CV) = (SD ÷ mean) × 100%. SD = Standard Deviation (σ), i.e., square root of the mean squared deviation.

Intra-Assay Precision (Within-run):

Using one batch kit, test low/medium/high-value samples 20 times consecutively. CV <9%.

Inter-Assay Precision (Between-run):

Using three kit batches, test low/medium/high-value samples 8 times each batch. CV <10%.

Stability:

When stored at recommended temperature within validity period, activity loss <5%. To minimize the impact of external factors on test results before and after kit degradation, laboratory conditions must maintain consistency — particularly in temperature control and incubation settings — while using the same operator reduces human error.

Disclaimer：

1.Our company solely guarantees the reagent kit quality. We shall not be held liable for sample consumption during use. Users must estimate required sample volumes in advance and reserve sufficient quantities. Final results depend on kit validity, operator techniques, and experimental conditions.

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2.Due to limitations in current scientific capabilities, comprehensive analysis of all raw materials is unfeasible. Users assume all risks for experimental failures. We shall not compensate for losses beyond the kit itself.

3.For samples not specified in the manual, conduct pre-experimental validation and preserve samples.

4.Tissue homogenates/cell lysates prepared with chemical lysates may cause ELISA deviations due to introduced compounds.

5.Factors including cell status, viability, density, and sampling time may prevent detection in culture supernatants.

6.Certain native/recombinant proteins (prokaryotic/eukaryotic) may be undetectable if incompatible with capture/detection antibodies.

7.Slight variations in detection limits, sensitivity, or color development time may occur between batches. Always follow the enclosed manual; online versions are for reference only.

8.Use only components supplied with this kit. Mixing products from other manufacturers is strictly prohibited. Strict adherence to protocols ensures optimal results.

9.Avoid exposure to strong light during storage/transport. Tighten all caps to prevent evaporation, microbial contamination, or reagent degradation.

10.Minor liquid in newly unpacked wells is normal and non-impactful. Remove strips from packaging immediately before use; do not unpack prematurely.

11.Variations in sample preparation or operational steps may alter results. Maintain stringent control at each stage for reproducibility.

12. Kits undergo rigorous pre-shipment inspection, but transport conditions or lab environments may cause result deviations or increased inter-assay CV.

13.This kit has not been validated against competitors' products or alternative methods. Result inconsistencies cannot be ruled out.

14.Antibodies are generated using recombinant antigens. Detection of other companies' recombinant proteins is not guaranteed due to differences in fragments, expression systems, or purification.

15.This kit may be unsuitable for samples with uncertain validity(e.g., gene knockout experiments).

16.For research purposes only. We shall not bear any legal responsibility for clinical diagnosis or other unauthorized applications.

Troubleshooting Guidance:

If experimental results are unsatisfactory, immediately photograph the color development results, preserve experimental data, retain the used microplate strips and unused reagents, fill out the after-sales service form (download and complete online), and contact our company's technical support for resolution. You may also refer to the following information.

Poor Standard Curve

Possible Cause	Recommended Action
Incorrect standard solution prep	Check pipettes/tips; confirm dilution accuracy.
Improper standard reconstitution	Centrifuge before opening; check for undissolved particles.
Standard degradation	Store/handle per recommendations.
Incomplete washing	Ensure adequate wash cycles, volume (300µl/well), and soak time.
Inappropriate curve scaling	Redraw with alternate scales.
Pipetting error	Use calibrated pipettes with proper technique.

No Signal

Possible Cause	Recommended Action
Target below detection range	Concentrate samples or reduce dilution factor.
Incompatible sample type	Validate unlisted samples with positive controls.
Buffer incompatibility	Verify assay buffer compatibility with target.
Improper sample prep	Follow correct prep/dilution protocols; confirm plate compatibility.
Insufficient antibodies	Test alternative antibody concentrations.
Low incubation temperature	Pre-equilibrate reagents to room temperature.
Incorrect wavelength	Confirm plate reader wavelength settings.
Over-aggressive washing	Adjust washer pressure; aspirate gently if manual.
Wells dried out	Seal plates during incubations to prevent drying.
Slow chromogen reaction	Prepare substrate fresh; extend incubation; check reagent integrity.

Possible Cause	Recommended Action
Improper kit equilibration	Pre-warm all reagents at room temperature for ≥20min.

High Background

Possible Cause	Recommended Action
Incomplete washing	Follow protocol wash cycles/volumes strictly.
Contaminated wash buffer	Prepare fresh wash buffer.
Excessive detection reagents	Verify dilution accuracy; reduce concentration if needed.
Ineffective blocking buffer	Test alternative blockers; add blocker to wash buffer.
Suboptimal salt concentration	Increase buffer salt concentration to reduce non-specific binding.
Delayed reading after stop solution	Read plate within 20min after adding stop solution.
Excessive antibody concentration	Titrate antibodies for optimal dilution.
Light exposure during substrate incubation	Incubate TMB in complete darkness.
Precipitate formation	Increase sample dilution or reduce substrate concentration.
Dirty plate bottom	Clean plate bottom before reading.
Expired/denatured reagents	Check kit expiration date; avoid expired reagents.
Incorrect incubation time/temp	Strictly follow recommended time/temperature.
Reused sealing film	Always use fresh sealing film.

Low Sensitivity

Possible Cause	Recommended Action
Improper kit storage	Store all components as recommended.
Insufficient target	Concentrate samples; reduce dilution.
Inactivated detection reagents	Verify enzyme/fluorophore activity.
Incorrect plate reader settings	Confirm correct wavelength (450nm) or exciayion/emission settings.
Poor target adsorption on plate	Covalently conjugate target to the microplate.
Incompatible sample types (e.g., serum and cell lysates)	Test with validated sample types; include positive controls.
Buffer/sample interference	Check for interfering compounds.
Mixed reagents from different kits	Use only reagents supplied in this kit.
Incomplete kit equilibration	Pre-warm all reagents at room temperature for ≥20min.

High Coefficient of Variation

Possible Cause	Recommended Action
Air bubbles in wells	Remove bubbles before reading.
Uneven/incomplete washing	Check washer nozzles; follow recommended wash methods.
Inadequate reagent mixing	Vortex/mix all reagents thoroughly.
Edge effects	Pre-equilibrate plates/reagents to room temperature.
Inconsistent sample prep/storage	Standardize prep; minimize freeze-thaw cycles; optimize storage.