（本试剂盒仅供体外研究使用，不用于临床诊断！）

# 兔心钠肽(ANP)酶联免疫吸附测定试剂盒
# 使用说明书

Rabbit ANP (Atrial Natriuretic Peptide) ELISA Kit

产品货号：E-EL-RB0002c

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：

| | |
|---|---|
| 全国免费电话 | 400-660-4808 |
| 销售部电话 | 027-87879180 |
| 技术部电话 | 027-87645690 |
| 电子邮箱（销售） | Perry@elabscience.cn |
| 电子邮箱（技术） | techsupport@elabscience.cn |
| QQ 客服 | 1037150941 |
| 网址 | www.elabscience.cn |

联系时请提供产品货号（见试剂盒标签），以便我们更高效地为您服务。

# 兔心钠肽(ANP)酶联免疫吸附测定试剂盒
# 使用说明书

产品货号：E-EL-RB0002c

（本试剂盒仅供体外研究使用、不用于临床诊断!）

**声明：**尊敬的客户，感谢您选用本公司的产品。本产品适用于体外定量检测兔血清、血浆或其它相关生物液体中天然和重组 ANP 浓度。**使用前请仔细阅读说明书并检查试剂组分！**如有疑问，请及时联系伊莱瑞特生物科技有限公司。

**试剂盒组成：**

| 中文名称 | 英文名称 | 规格 | 保存条件 |
|---|---|---|---|
| ELISA 酶标板（可拆卸） | Micro ELISA Plate(Dismountable) | 8×12 / 8×6 * | 4℃/-20℃ # |
| 冻干标准品 | Reference Standard | 2/1 支* | 4℃/-20℃ # |
| 标准品&样品稀释液 | Reference Standard & Sample Diluent | 1 瓶 20mL/12mL * | 4℃ |
| 浓缩生物素化抗体 | Concentrated Biotinylated Detection Ab | 1 支 120μL/70μL * | 4℃/-20℃ # |
| 生物素化抗体稀释液 | Biotinylated Detection Ab Diluent | 1 瓶 10mL/6mL * | 4℃ |
| 浓缩 HRP 酶结合物 | Concentrated HRP Conjugate | 1 支 120μL/70μL * | 4℃(避光) |
| 酶结合物稀释液 | HRP Conjugate Diluent | 1 瓶 10mL/6mL * | 4℃ |
| 浓缩洗涤液（25×） | Concentrated Wash Buffer (25×) | 1 瓶 30mL/16mL * | 4℃ |
| 底物溶液（TMB） | Substrate Reagent | 1 瓶 10mL/6mL * | 4℃(避光) |
| 反应终止液 | Stop Solution | 1 瓶 10mL/6mL * | 4℃ |
| 封板覆膜 | Plate Sealer | 5/3 张* | |
| 产品说明书 | Product Description | 1 份 | |
| 质检报告 | Certificate of Analysis | 1 份 | |

**特别说明：**

*: [96T/48T]（打开包装后请及时检查所有物品是否齐全完整）

#: 一周内使用可存于4℃，需长时间存放或多次使用建议存于-20℃.

相关试剂在分装时会比标签上标明的体积稍多一些，请在使用时量取而非直接倒出！

**检测原理：**

本试剂盒采用竞争ELISA法。用ANP抗原包被于酶标板上，实验时样品或标准品中的ANP与包被的ANP竞争生物素标记的抗ANP单抗上的结合位点，游离的成分被洗去。加入辣根过氧化物酶标记的亲和素，生物素与亲和素特异性结合而形成免疫复合物，游离的成分被洗去。加入显色底物(TMB)，TMB在辣根过氧化物酶的催化下呈现蓝色，加终止液后变成黄色。用酶标仪在450nm波长处测OD值，ANP浓度与$OD_{450}$值之间呈反比，通过绘制标准曲线计算出样品中ANP的浓度。

**样品收集：**

1. 血清：全血样品于室温放置2小时或4℃过夜后于1000×g离心20分钟，取上清即可检测，收集血液的试管应为一次性的无热原，无内毒素试管。
2. 血浆：抗凝剂推荐使用EDTA-Na2，样品采集后30分钟内于1000×g离心15分钟，取上清即可检测。避免使用溶血，高血脂样品。
3. 组织匀浆：用预冷的PBS (0.01M, pH=7.4)冲洗组织，去除残留血液（匀浆中裂解的红细胞会影响测量结果），称重后将组织剪碎。将剪碎的组织与对应体积的PBS（一般按1:9的重量体积比，比如1g的组织样品对应9mL的PBS，具体体积可根据实验需要适当调整，并做好记录。推荐在PBS中加入蛋白酶抑制剂）加入玻璃匀浆器中，于冰上充分研磨。为了进一步裂解组织细胞，可以对匀浆液进行超声破碎，或反复冻融。最后将匀浆液于5000×g离心5~10分钟，取上清检测。
4. 细胞培养上清：取细胞培养上清于1000×g离心20分钟，除去杂质及细胞碎片。取上清检测。
5. 其它生物样品：1000×g离心20分钟，取上清即可检测
   具体处理方法可参考：http://www.elabscience.cn/index.php/resources/view/aid/17671.jsp
6. 样品应清澈透明，悬浮物应离心去除。
7. 样品收集后若在1周内进行检测的可保存于4℃，若不能及时检测，请按一次使用量分装，冻存于-20℃(1个月内检测)，或-80℃（6个月内检测），避免反复冻融。
8. 如果您的样品中检测物浓度高于标准品最高值，请根据实际情况，做适当倍数稀释（**建议先做预实验，以确定稀释倍数**）。

**试验所需自备物品:**

1. 酶标仪(450nm波长滤光片)
2. 高精度移液器，EP管及一次性吸头：0.5-10μL, 2-20μL, 20-200μL, 200-1000μL
3. 37℃恒温箱，双蒸水或去离子水
4. 吸水纸

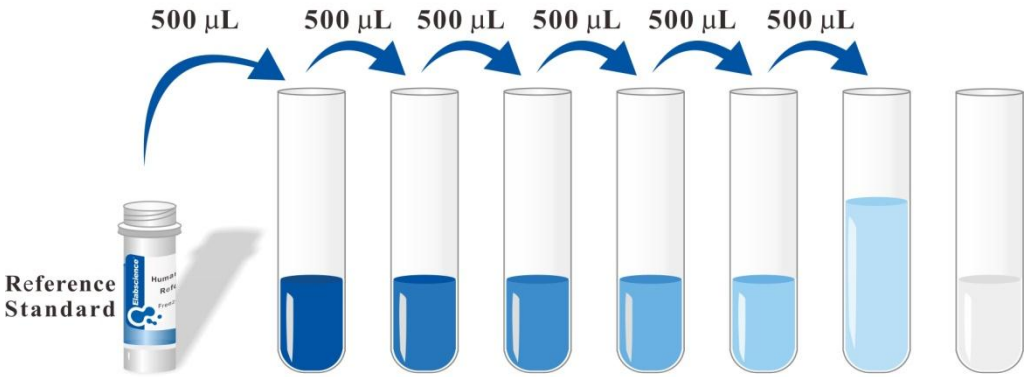
**检测前准备工作:**

1. 请提前20分钟从冰箱中取出试剂盒，平衡至室温。
2. 将浓缩洗涤液用双蒸水稀释**(1:25)**。未用完的放回4℃。从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象，可用40℃水浴微加热使结晶完全溶解后再配制洗涤液（加热温度不要超过50℃，使用时洗涤液应为室温）。当日使用
3. 标准品: 于10000×g离心1分钟，加入标准品&样品稀释液**1.0mL**至冻干标准品中，旋紧管盖，静置10分钟，上下颠倒数次，待其充分溶解后，用移液器将其轻轻混匀(浓度为**1000pg/mL**)。然后根据需要进行倍比稀释**（注：不要直接在反应孔中进行倍比稀释）**。建议配制成以下浓度：**1000、500、250、125、62.5、31.25、15.625、0pg/mL**，标准品&样品稀释液直接作为空白孔0pg/mL。如配制500pg/mL标准品：取0.5mL1000pg/mL的上述标准品加入含有0.5mL标准品&样品稀释液的EP管中，混匀即可，其余浓度依此类推。
4. 生物素化抗体工作液：实验前计算当次实验所需用量（以50μL/孔计），实际配制时应多配制100-200μL。使用前15分钟，以生物素化抗体稀释液稀释浓缩生物素化抗体**(1:100)**成工作

浓度。当日使用。

**5.** 酶结合物工作液：实验前计算当次实验所需用量（以100μL/孔计），实际配制时应多配制100-200μL。使用前15分钟，以酶结合物稀释液稀释浓缩HRP酶结合物**(1:100)**成工作浓度。当日使用。

**标准品稀释方法图例：**（以500μL/管为例，也可根据实际用量来稀释，如200μL/管）




| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Tube |
|---|---|---|---|---|---|---|---|---|
| 1000 | 500 | 250 | 125 | 62.5 | 31.25 | 15.625 | 0 | pg/mL |

**洗涤方法:**

1. 自动洗板机：每孔加入洗涤液350μL，注入与吸出间隔60秒。
2. 手工洗板：甩尽孔内液体，在洁净的吸水纸上拍干，每孔加洗涤液350μL，浸泡1-2分钟，吸去（不可触及板壁）或甩掉酶标板内的液体，在厚的吸水纸上拍干。

**操作步骤:**

实验开始前，各试剂均应平衡至室温；试剂或样品配制时，均需充分混匀，并尽量避免起泡。

1. 加样：分别设空白孔、标准孔、待测样品孔。空白孔加标准品&样品稀释液 50μL，余孔分别加**标准品**或待测样品 50μL，立即每孔加入配好的**生物素化抗体工作液** 50μL（在使用前 15 分钟内配制），注意不要有气泡，加样时将样品加于酶标板底部，尽量不触及孔壁，轻轻晃动混匀。给酶标板覆膜，37℃孵育 45 分钟。**为保证实验结果有效性，每次实验请使用新的标准品溶液。**
2. 弃去孔内液体，甩干，洗板 3 次，每次浸泡 1-2 分钟，大约 350μL /每孔，甩干并在吸水纸上轻拍将孔内液体拍干。
3. 每孔加**酶结合物**工作液（临用前 15 分钟内配制）100μL，加上覆膜，37℃温育 30 分钟。
4. 弃去孔内液体，甩干，洗板 5 次，方法同步骤 3。
5. 每孔加**底物溶液(TMB)**90μL，酶标板加上覆膜 37℃避光孵育 15 分钟左右（根据实际显色情况酌情缩短或延长，但不可超过 30 分钟。当标准孔出现明显梯度时，即可终止）。
6. 每孔加**终止液** 50μL，终止反应，此时蓝色立转黄色。终止液的加入顺序应尽量与底物液的加入顺序相同。

7. 立即用酶标仪在 450nm 波长测量各孔的光密度（OD 值）。应提前打开酶标仪电源，预热仪器，设置好检测程序。
8. 实验完毕后将未用完的试剂按规定的保存温度放回冰箱保存至有效期结束。

**注意事项：**

1. **保存：**试剂盒中各试剂请按说明书提示合理存放。在储存及温育过程中避免将试剂暴露在强光中。所有试剂瓶盖须旋紧以防止蒸发和微生物的污染，否则可能会出现错误的结果。
2. **酶标板：**刚开启的酶标板孔中可能会有少许水样物质，此为正常现象，不会对实验结果造成任何影响。暂时不用的板条应拆卸后放入备用铝箔袋，按推荐温度存放！
3. **加样：**加样或加试剂时，第一个孔与最后一个孔的加样时间间隔如果太大，将会导致不同的“预温育”时间，从而明显地影响到测量值的准确性及重复性。每次的加样时间最好控制在10分钟内。推荐设置复孔。
4. **温育：**为防止样品蒸发，实验时必须给酶标板覆膜；洗板后应尽快进行下步操作，避免酶标板处于干燥状态；严格遵守给定的温育时间和温度。
5. **洗涤**：洗涤过程中反应孔中残留的洗涤液应在吸水纸上拍干，勿将滤纸直接放入反应孔中吸水。在读数前要注意清除底部残留的液体和手指印，以免影响酶标仪读数。
6. **试剂配制：Concentrated Biotinylated Detection Ab**及**Concentrated HRP Conjugate**体积较小，运输过程会使液体沾到管壁或瓶盖，因此使用前1000转/分离心1min，以使附着管壁或瓶盖的液体沉积到管底。取用前，请用移液器小心吹打4-5次使溶液混匀。标准品、生物素化抗体工作液、酶结合物工作液请根据所需用量配制，并使用相应的稀释液配制，不能混淆。请精确配制标准品及工作液，尽量不要微量配制（如吸取**Concentrated Biotinylated Detection Ab**时，一次不要小于10μL），以避免由于不准确稀释而造成浓度误差；请勿重复使用已稀释过的标准品、生物素化抗体工作液、酶结合物工作液。若需要分次使用标准品应按照每一次用量分装，将其放在-20～-80℃贮存。避免反复冻融。
7. **显色时间的控制：**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），如梯度已很明显，请提前加入终止液终止反应，避免颜色过深影响酶标仪读数。
8. **底物：**底物请避光保存，在储存和温育时避免强光直接照射。
9. **混匀：**充分轻微混匀对反应结果尤为重要，最好使用微量振荡器(使用最低频率)，如无微量振荡器，可在反应前手工轻轻敲击酶标板框混匀。
10. **安全：**试验中请穿着实验服并带乳胶手套做好防护工作。特别是检测血液或者其他体液样品时，请按国家生物试验室安全防护条例执行。
11. 不同批号的试剂盒组份不能混用（洗涤液和反应终止液除外）
12. 试验中所用的EP管和吸头均为一次性使用，**严禁混用，否则将影响试验结果！**

**结果判断:**

1. 以标准品的浓度为横坐标，OD值为纵坐标，绘制标准曲线。如有设置复孔，则应取其平均值计算。以标准品的浓度为横坐标，OD值为纵坐标，绘出标准曲线。亦可以OD值为横坐标，标准品的浓度为纵坐标，绘出标准曲线。
2. 推荐使用专业的曲线制作软件，如curve expert 1.3或1.4，在软件界面既可根据样品OD值，由标准曲线查出相应的浓度，乘以稀释倍数；亦可将样品的OD值代入标准曲线的拟合方程式，计算出样品浓度，再乘以稀释倍数，即为样品的实际浓度。
3. 若样品OD值高于标准曲线上限，应适当稀释后重测，计算浓度时应乘以稀释倍数。

**灵敏度、检测范围、特异性和重复性:**

- 灵敏度：最小可测 9.375pg/mL。
- 检测范围：15.625–1000pg/mL。
- 特异性：可检测重组或天然的兔 ANP，且与其它相关蛋白无交叉反应。
- 重复性：板内，板间变异系数均<10%。

**操作概要**

1. 在各孔中加入标准品或样品各 50μL
2. 立即加入 50μL 生物素化抗体工作液，37℃孵育 45 分钟
3. 洗涤 3 次
4. 加入 100μL 酶结合物工作液， 37℃孵育 30 分钟
5. 洗涤 5 次
6. 加入 90μL 底物溶液， 37℃孵育 15 分钟左右
7. 加入 50μL 终止液，立即在 450nm 波长处测量 OD 值
8. 结果计算

## 问题分析

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

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

## 声明

1. 限于现有条件及科学技术水平，尚不能对所有原料进行全面的鉴定分析，本产品可能存在一定的质量技术风险。
2. 最终的实验结果与试剂的有效性、实验者的相关操作以及当时的实验环境密切相关，请务必准备充足的待测样品。
3. 只有全部使用试剂盒内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 Elabscience 的实验说明才会得到最佳的检测结果。
4. 有效期：6 个月。
5. 本操作说明同样适用于 48T 试剂盒。

# Rabbit ANP (Atrial Natriuretic Peptide) ELISA Kit

## Product Manual

Catalog No: E-EL-RB0002c

（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGONOSIS !）

Dear customer, Thank you for choosing our products. Please read the instructions carefully before use and check all the reagent compositions! If in doubt, please contact Elabscience.

### Kit Components:

| Item | Specifications | Storage |
|---|---|---|
| Micro ELISA Plate(Dismountable) | 8×12 or 8×6 * | 4℃/-20℃ # |
| Reference Standard | 2/1vial * | 4℃/-20℃ # |
| Reference Standard & Sample Diluent | 1vial 20mL/12mL * | 4℃ |
| Concentrated Biotinylated Detection Ab | 1vial 120μL /70μL* | 4℃/-20℃ # |
| Biotinylated Detection Ab Diluent | 1vial 10mL/6mL* | 4℃ |
| Concentrated HRP Conjugate | 1vial 120μL/70μL * | 4℃(shading light) |
| HRP Conjugate Diluent | 1vial 10mL/6mL * | 4℃ |
| Concentrated Wash Buffer (25×) | 1vial 30mL/16mL * | 4℃ |
| Substrate Reagent | 1vial 10mL/6mL * | 4℃(shading light) |
| Stop Solution | 1vial 10mL/6mL * | 4℃ |
| Plate Sealer | 5/3pieces * | |
| Product Description | 1 copy | |
| Certificate of Analysis | 1 copy | |

**Note:**

*: [96T/48T]

#: It's OK to keep the kit in 4℃, if the kit is scheduled to be used up in one week. Please keep the reagent in -20℃ for long-term storage or repeated use.

The reagent in each vial is slightly more that its volume written on label, please take out the required volume by certain tools (such as transferpettor, measuring cylinder), rather than pouring directly.

### Test principle

This ELISA kit uses Competitive-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with ANP. During the reaction, ANP in the sample or standard competes with a fixed amount of ANP on the solid phase supporter for sites on the Biotinylated Detection Ab specific to ANP. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ±2 nm. The concentration of ANP in the samples is then determined by comparing the O.D. of the samples to the standard curve.

**Sample collection and storage**

**Serum** - Allow samples to clot for 2 hours at room temperature or overnight at 4℃ before centrifugation for 20 minutes at approximately 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

**Plasma** - Collect plasma using EDTA-Na2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8℃ within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

**Tissue homogenates–** For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate.

**Cell culture supernate –** Centrifuge supernate for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8℃. Collect the clear supernate and carry out the assay immediately.

**Other biological fluids –**Centrifuge samples for 20 minutes at 1000×g at 2 - 8℃. Collect the supernatant and carry out the assay immediately. (You can refer to our website for detailed processing method: http://www.elabscience.cn/index.php/resources/view/aid/17671.jsp)

**Sample preparation –** Samples should be clear and transparent and be centrifuged to remove suspended solids.

**Note:** Serum and plasma to be used within 7 days when stored at 2-8℃, otherwise samples must be divided and stored at -20℃ (≤1 month) or -80℃ (≤6 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature. If the sample concentration is higher than the maximum standard value, please dilute it with appropriate factor according to the actual situation. (A pre-test is recommended to determine the dilute factor).

**Other supplies required**

Microplate reader with 450nm wavelength filter

High-precision transferpettor, EP tubes and disposable pipette tips

37℃ Incubator, Deionized or distilled water

Absorbent paper

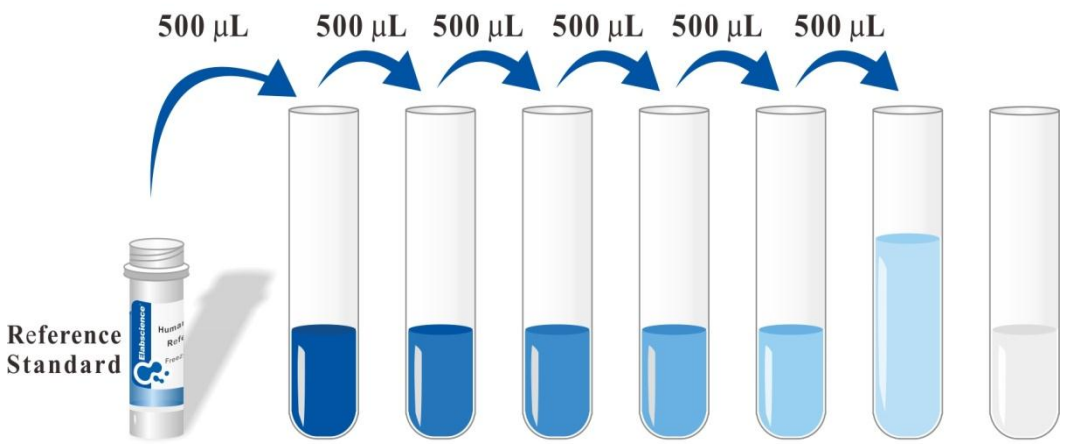
**Reagent preparation**

Bring all reagents to room temperature before use.

**Wash Buffer -** Dilute 30mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4℃. If crystals have formed in the concentrate, you can warm it with 40℃ water bath (Heating temperature should not exceed 50℃) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

**Standard-**Centrifuge at 10,000×g for 1 minute, and reconstitute the Standard with **1.0mL** of Reference Standard&Sample Diluent. Tighten the lid, let it stand for 10minutes and turn it upside down for several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a stock solution of 1000pg/mL. Then make serial dilutions as needed (Making serial dilution in the wells directly is not permitted). The recommended concentrations are as follows:**1000、500、250、125、62.5、31.25、15.625、0pg/mL** . As if you want to make standard solution at the concentration of 500pg/mL, you can take 0.5mL the standard at 1000pg/mL, add it to an EP tube with 0.5mL Reference Standard &Sample Diluent, and mix it. The procedures of making the remaining concentrations are all the same. The undiluted standard serves as the highest standard (1000pg/mL). The Reference Standard &Sample Diluent serves as the zero (0pg/mL).

(500μL/tube，for example. Can also be diluted according to the actual amount，such as 200μL/tube)




| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Tube |
|---|---|---|---|---|---|---|---|---|
| 1000 | 500 | 250 | 125 | 62.5 | 31.25 | 15.625 | 0 | pg/mL |

**Biotinylated Detection Ab —**Calculate the required amount before experiment (50μL/well). In actual preparation you should prepare 100~200μL more. Dilute the concentrated Biotinylated Detection Ab to the working concentration using Biotinylated Detection Ab Diluent(1:100).

**Concentrated HRP Conjugate —** Calculate the required amount before experiment (100μL/well). In actual preparation you should prepare 100~200μL more. Dilute the Concentrated HRP Conjugate to the working concentration using Concentrated HRP Conjugate Diluent (1:100).

**Washing Procedure:**

1. **Automated washer:** add 350μL wash buffer into each well, the interval between injection and suction should be set about 60s.
2. **Manual wash:** add 350μL wash buffer into each well, soak it for 1~2minutes, suck (no inside wall touching) or get rid of liquid within the micro ELISA plate and pat it dry on thick clean absorbent paper.

**Assay procedure**

Allow all reagents to reach room temperature **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.**

1. **Add Sample and Biotinylated Detection Ab:** Add 50μL of Standard, Blank, or Sample per well. The blank well is added with Reference Standard &Sample Diluent. Immediately add 50 μL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer we provided. Gently tap the plate to ensure thorough mixing. Incubate for 45minutes at 37℃. (Solutions are added to the bottom of micro ELISA plate well, avoid inside wall touching and foaming to the best of your ability.)
2. **Wash:** Aspirate each well and wash, repeating the process three times Wash by filling each well with Wash Buffer (approximately 350μL) using a squirt bottle, multi-channel pipette, manifold dispenser or automated washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.
3. **HRP Conjugate:** Add 100μL of HRP Conjugate working solution to each well. Cover with a new Plate sealer. Incubate for 30minutes at 37℃.
4. **Wash:** Repeat the aspiration/wash process for five times as conducted in step 4.
5. **Substrate:** Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for about 15 minutes at 37℃. Protect from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30minutes. When apparent gradient appeared in standard wells, you can terminate the reaction.
6. **Stop**: Add 50μL of Stop Solution to each well. Color turn to yellow immediately. The adding order of stop solution should be as the same as the substrate solution.
7. **OD Measurement**: Determine the optical density (OD value) of each well at once, using a microplate reader set to 450 nm. You should open the microplate reader ahead, preheat the instrument, and set the testing parameters.
8. After experiment, put all the unused reagents back into the refrigerator according to the specified storage temperature respectively until their expiry.

**Important Note:**

1. **Storage**: All the reagents in the kit should be stored following the instructions. Exposure of reagents to strong light should be avoided in the process of incubation and storage. All the taps of reagents should be tightened to prevent evaporation and microbial contamination, or erroneous results may occur.
2. **ELISA Plate**: Little water-like substance may appear in the ELISA Plate just opened, this is normal and will not have any impact on the experiment results. Keep the remaining plates in spare aluminum foil bag, and keep it in temperature suggested before.
3. **Add Sample**: The interval of sample adding between the first well and the last well should not be too long, otherwise will cause different pre-incubation time, which will significantly affect the experiment's accuracy and repeatability. The interval controlled within 10minutes is good. Parallel measurement is recommended.
4. **Incubation**: To prevent evaporation, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Do not let the strips dry at any time during the assay. Strict compliance with the given incubation time and temperature.
5. **Washing**: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings Residual liquid in the reaction wells should be pat dry against absorbent paper in the washing process. But don't put absorbent paper into reaction wells directly. Note that clear the residual liquid and fingerprint in the bottom before measurement, so as not to affect the microtiter plate reader.
6. **Reagent Preparation**: As the volume of Concentrated Biotinylated Detection Ab and Concentrated HRP Conjugate is very small, liquid may adhere to the tube wall or tube cap when being transported. You better hand-throw it or centrifugal it for 1 minute at 1000rpm. Please pipette the solution for 4-5 times before pippeting. Please carefully reconstitute Standards, working solutions of Biotinylated Detection Ab and HRP Conjugate according to the instructions. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10μL for once pipetting. Do not reuse standard solution, working solution of Biotinylated Detection Ab and HRP Conjugate, which have been diluted. If you need to use standard repeatedly, you can divide the standard into small pack according to the amount of each assay, keep them at -20～-80℃ and avoid repeated freezing and thawing
7. **Reaction Time Control:** Please control reaction time strictly following this product description!
8. **Substrate:** Substrate Solution is easily contaminated. Please protect it from light.
9. **Mixing:** You'd better use microoscillator at the lowest frequency, as sufficient and gentle mixing is particularly important to reaction result. If there is no microsocillator available, you can knock the ELISA plate frame gently with your finger before reaction.
10. **Security:** Please wear lab coats and latex gloves for protection. Especially detecting samples of blood or other body fluid, please perform following the national security columns of biological laboratories.

11. Do not use component from different batches of kit (washing buffer and stop solution can be an exception).
12. To avoid cross-contamination, change pipette tips between adding of each standard level, between sample adding, and between reagent adding. Also, use separate reservoirs for each reagent. **Otherwise, the results will be inaccurate!**

**Calculation of results**

Average the duplicate readings for each standard and samples. Create a standard curve by plotting the mean OD value for each standard on the y-axis or x-axis against the concentration on the x-axis or y-axis and draw a best fit curve through the points on the graph. It is recommended to use some professional software to do this calculation, such as curve expert 1.3 or 1.4. In the software interface, a best fitting equation of standard curve will be calculated using OD values and concentrations of standard sample. The software will calculate the concentration of samples after entering the OD value of samples. Also, you can enter the corresponding fitting equation and OD value of samples into Excel to get the concentration of samples. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor.

**Sensitivity**

The minimum detectable dose of ANP is 9.375pg/mL (The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero).

**Detection Range**

15.625–1000pg/mL.

**Specificity**

This kit recognizes recombinant and natural Rabbit ANP. No significant cross-reactivity or interference was observed.

**Repeatability:**

Coefficient of variation were<10%

## SUMMARY

1. Add 50μL standard or sample to each well
2. Immediately add 50μL Biotinylated Detection Ab to each well
3. Incubate for 45 minutes at 37℃
4. Aspirate and wash 3 times
5. Add 100μL HRP Conjugate to each well. Incubate for 30 minutes at 37℃
6. Aspirate and wash 5 times
7. Add 90μL Substrate Reagent. Incubate 15 minutes at 37℃
8. Add 50μL Stop Solution. Read at 450nm immediately
9. Calculation of results

**Troubleshooting**

If the results are not good, please take pictures, keep the used plate and remaining reagents. Then contact our technical department. Meanwhile, you could refer to the following materials.

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

**Declaration:**

1. Limited by the current conditions and scientific technology, we can't completely conduct the comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. The final experimental results will be closely related to validity of the products, operation skills of the operators and the experimental environments. Please make sure that sufficient samples are available.
3. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions in the description!
4. Valid period: 6 months.
5. This description is also suitable for 48T kit.

Copyright ©2014-2015 Elabscience Biotechnology Co.,Ltd All Rights Reserved