小鼠脂联素（APN/ADP)酶联免疫分析

试剂盒使用说明书

96T

使用目的

本试剂盒用于测定小鼠血清、血浆及相关液体样本中脂联素（APN/ADP)含量。

实验原理

本试剂盒应用双抗体夹心法测定标本中小鼠脂联素（APN/ADP)水平。用纯化的小鼠脂联素（APN/ADP)抗体包被微孔板，制成固相抗体，往包被单抗的微孔中依次加入脂联素（APN/ADP)，再与 HRP 标记的脂联素（APN/ADP)抗体结合，形成抗体-抗原-酶标抗体复合物，经过彻底洗涤后加底物 TMB 显色。TMB 在 HRP 酶的催化下转化成蓝色，并在酸的作用下转化成最终的黄色。颜色的深浅和样品中的脂联素（APN/ADP)呈正相关。用酶标仪在 450nm 波长下测定吸光度（OD 值），通过标准曲线计算样品中小鼠脂联素（APN/ADP)浓度。

试剂盒提供的材料

1	30 倍浓缩洗涤液	20ml×1 瓶	7	终止液	6ml×1 瓶
2	酶标试剂	6ml×1 瓶	8	标准品（240μg/L）	0.5ml×1 瓶
3	酶标包被板	12 孔×8 条	9	标准品稀释液	1.5ml×1 瓶
4	样品稀释液	6ml×1 瓶	10	说明书	1 份
5	显色剂 A 液	6ml×1 瓶	11	封板膜	2 张
6	显色剂 B 液	6ml×1/瓶	12	密封袋	1 个

实验需准备的材料设备

1、酶标仪
2、移液器及枪头
3、洗板机
4、试管、离心管、量筒等
5、蒸馏水或去离子水
6、电热恒温箱

样本的收集

液体样本的收集

1、血清：用无菌管收集，室温血液自然凝固 10-20 分钟，2-8℃条件离心 20 分钟左右（2000-3000 转/分），仔细收集上清，保存过程中如出现沉淀，应再次离心。

2、血浆：应根据标本的要求选择 EDTA、肝素钠或柠檬酸钠作为抗凝剂，混合 10-20 分钟后，2-8℃条件离心 20 分钟左右（2000-3000 转/分），仔细收集上清，保存过程中如有沉淀形成，应该再次离心。

3、尿液：用无菌管收集，2-8℃条件离心 20 分钟左右（2000-3000 转/分）。仔细收集上清，保存过程中如有沉淀形成，应再次离心。

4、胸腹水：用无菌管收集，2-8℃条件离心 20 分钟左右（2000-3000 转/分），仔细收集上清，保存过程中如有沉淀形成，应再次离心。

5、脑脊液：用无菌管收集，2-8℃条件离心 20 分钟左右（2000-3000 转/分），仔细收集上清，保存过程中如有沉淀形成，应再次离心。

6、唾液：用无菌管收集，2-8℃条件离心 20 分钟左右（2000-3000 转/分），仔细收集上清，保存过程中如有沉淀形成，应再次离心。

7、细胞培养上清：检测分泌性的成份时，用无菌管收集。2-8℃条件离心 20 分钟左右（2000-3000 转/分），仔细收集上清，保存过程中如有沉淀形成，应再次离心。

8、牛奶：用无菌管收集，2-8℃条件离心 20 分钟左右（2000-3000 转/分），仔细收集上清，保存过程中如有沉淀形成，应再次离心。

9、蜂蜜：用无菌管收集，2-8℃条件离心 20 分钟左右（2000-3000 转/分），仔细收集上清，保存过程中如有沉淀形成，应再次离心。

10、全血：用含有抗凝剂的无菌管收集，立即轻轻摇动，来回轻轻颠倒数次，使血液和抗凝剂混匀，以防血液凝固。

固体样本的收集

1、组织标本：切割标本后，称取 1g 组织，加入 9ml 的 pH7.2-7.4 左右的 PBS，用手工或匀浆器将标本匀浆充分。离心 20 分钟左右（2000-3000 转/分），仔细收集上清。分装一份待检测，其余冷冻备用，保存过程中如有沉淀形成，应再次离心。对于植物组织，不好匀浆的话，就在液氮中充分研磨。

2、细胞内蛋白样本：许多待测蛋白不是分泌蛋白，检测细胞内的蛋白，需要先收集细胞，洗涤干净，再用超声破碎细胞，离心取上清。

（1）培养的细胞

A、动物细胞：用 PH7.2-7.4 的 PBS 稀释细胞悬液，使细胞浓度达到 100 万/ml 左右。通过超声波破碎，以使细胞破坏并放出细胞内成份。2-8℃条件离心 20 分钟左右（2000-3000 转/分），仔细收集上清，保存过程中如有沉淀形成，应再次离心。

B、植物细胞：用 PH7.2-7.4 的 PBS 稀释细胞悬液，使细胞浓度达到 100 万/ml 左右，置于冰盒上，用超声破碎仪，设置破碎 2s，冷却 30s 的方式，充分破碎细胞，使细胞破坏并放出细胞内成份。2-8℃条件离心 20 分钟左右（2000-3000 转/分），仔细收集上清，保存过程中如有沉淀形成，应再次离心。

（2）组织的细胞

切割标本后，称取 1g 组织，加入 9ml 的 pH7.2-7.4 左右的 PBS，用手工或匀浆器将标本匀浆充分。2-8℃条件离心 20 分钟左右（2000-3000 转/分），去除上清，再用 pH7.2-7.4 左右的 PBS 小心洗涤沉淀的细胞三遍。再用上述的细胞破碎方法破碎细胞。

3、咽拭子：加入 2ml 的 pH7.2-7.4 左右的 PBS，溶解咽拭子头部，摇匀，用镊子取出咽拭子并挤干液体，2-8℃条件离心 20 分钟左右（2000-3000 转/分），仔细收集上清。分装一份待检测，其余冷冻备用，保存过程中如有沉淀形成，应再次离心。如果是测分泌蛋白，直接取上清检测，测试细胞内蛋白，要破碎细胞。

4、植物标本：

A、样本在保持鲜重的同时，每个样本重量不能低于 50mg；

B、组织匀浆比例按照 10%进行，即 1g 组织加 9mL 匀浆液，匀浆液用 PBS，浓度是 0.01mol/l，PH 值控制在 7.2-7.4。样本重量如有调整，相对应匀浆液按照 1:9 的比例调整即可；

C、剪碎叶片组织放入碾钵，用液氮碾磨成粉末，加入换算后匀浆液的量；

D、离心取上清，4000-5000 转每分钟，时间为 15 分钟。

样本的要求

1、标本采集后尽早进行提取，提取按相关文献进行，提取后应尽快进行实验。若不能马上进行试验，可将标本放于-20℃保存，但应避免反复冻融。

2、不能检测含 NaN3 的样品，因 NaN3 抑制辣根过氧化物酶的（HRP）活性。

3、以上是通用的样本处理方法，无法涵盖各种样本，对于一些特殊样本，建议实验人员多参考已发表的文献，自行设计合理的样本处理方法。

检测步骤

1、标准品的稀释：本试剂盒提供原倍标准品一支，用户可按照下列图表在小试管中进行稀释。

120μg/L	5 号标准品	150μl 的原倍标准品加入 150μl 标准品稀释液
60μg/L	4 号标准品	150μl 的 5 号标准品加入 150μl 标准品稀释液
30μg/L	3 号标准品	150μl 的 4 号标准品加入 150μl 标准品稀释液
15μg/L	2 号标准品	150μl 的 3 号标准品加入 150μl 标准品稀释液
7.5μg/L	1 号标准品	150μl 的 2 号标准品加入 150μl 标准品稀释液

2.加样：分别设空白孔（空白对照孔不加样品及酶标试剂，其余各步操作相同）、标准孔、待测样品孔。在酶标包被板上标准品准确加样 50μl，待测样品孔中先加样品稀释液 40μL，然后再加待测样品 10μl（样品最终稀释度为 5 倍）。加样将样品加于酶标板孔底部，尽量不触及孔壁，轻轻晃动混匀。

3.温育：用封板膜封板后置 37℃温育 30 分钟。

4.配液：将 30 倍浓缩洗涤液用蒸馏水 30 倍稀释后备用。

5.洗涤：小心揭掉封板膜，弃去液体，甩干，每孔加满洗涤液，静置 30 秒后弃去，如此重复 5 次，拍干。

6.加酶：每孔加入酶标试剂 50μl，空白孔除外。

7.温育：操作同 3。

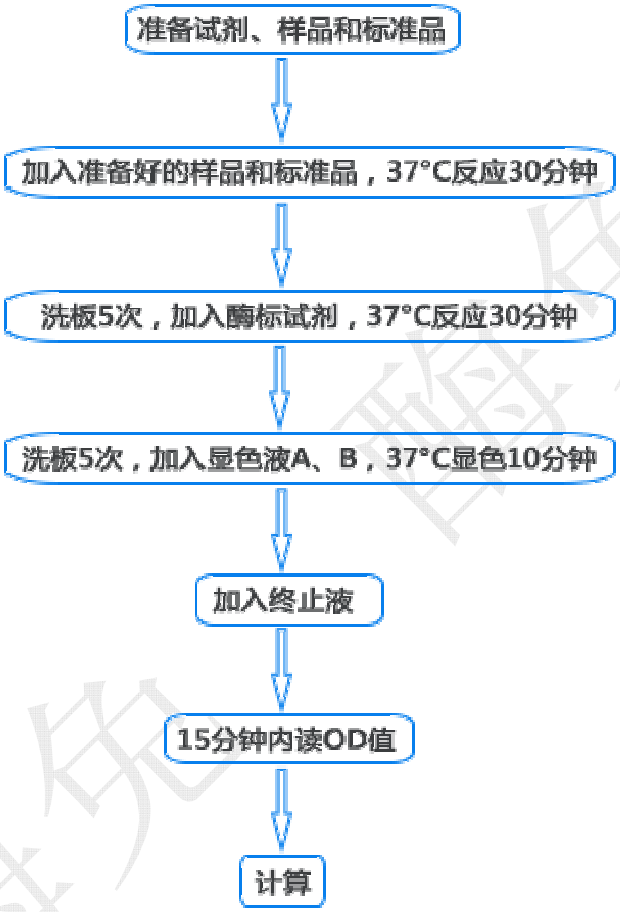
8.洗涤：操作同 5。

9.显色：每孔先加入显色剂 A50μl，再加入显色剂 B50μl，轻轻震荡混匀，37℃避光显色 10 分钟。

10.终止：每孔加终止液 50μl，终止反应（此时蓝色立转黄色）。

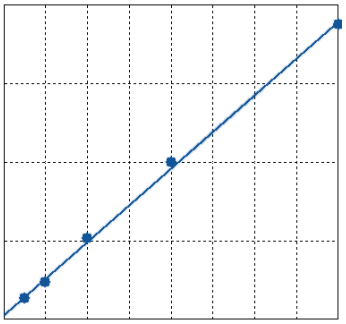
11.测定：以空白孔调零，450nm 波长依序测量各孔的吸光度（OD 值）。 测定应在加终止液后 15 分钟以内进行。

检测步骤概要



结果计算

以标准物的浓度为横坐标，OD 值为纵坐标，在坐标纸上绘出标准曲线，根据样品的 OD 值由标准曲线查出相应的浓度；再乘以稀释倍数；或用标准物的浓度与 OD 值计算出标准曲线的直线回归方程式，将样品的 OD 值代入方程式，计算出样品浓度，再乘以稀释倍数，即为样品的实际浓度。



此图仅供参考

性能参数

1、样品线性回归与预期浓度相关系数 R 值为 0.95 以上
2、批内变异系数与批间变异系数应分别小于 10%和 12%

检测范围

3μg/L -120μg/L

保存条件及有效期

1、试剂盒保存： 2-8℃
2、有效期： 6 个月

注意事项

1、本试剂盒用于科学研究，非诊断试剂，不能用于临床诊断。
2、请在本试剂盒标记的有效期内使用。
3、试剂盒的试剂不能与其他批号的试剂或其他来源的试剂混合使用。
4、试剂盒从冷藏环境中取出应在室温平衡 1 小时后方可使用，酶标包被板开封后如未用完，板条应装入密封袋中保存。
5、任何标准品稀释、操作人员、移液技术、洗涤技术、孵育温度、试剂盒保存时间的改变，都将影响结合反应。
6、本试剂盒在设计上去除或降低了生物学样本中的一些内源性干扰因素，并非所有可能的影响因素都已经去除。
7、浓缩洗涤液可能会有结晶析出，稀释时可在水浴中加温助溶，洗涤时不影响结果。
8、各步加样均应使用加样器，并经常校对其准确性，以避免试验误差。一次加样时间最好控制在 5 分钟内，如标本数量多，推荐使用排枪加样。
9、请每次测定的同时做标准曲线，最好做复孔。如标本中待测物质含量过高（样本 OD 值大于标准品孔第一孔的 OD 值），请先用样品稀释液稀释一定倍数（n 倍）后再测定，计算时请最后乘以总稀释倍数（×n×5）。
10、封板膜只限一次性使用，以避免交叉污染。
11、底物请避光保存。
12、严格按照说明书的操作进行，试验结果判定必须以酶标仪读数为准。
13、所有样品，洗涤液和各种废弃物都应按传染物处理。
14、本试剂不同批号组分不得混用。

Mouse APN/ADP

FOR RESEARCH USE ONLY

96 determinations

Purpose

This kit allows for the determination of APN/ADP concentrations in Mouse serum, plasma and other biological fluids

Principle of the assay

The kit assay Mouse APN/ADP level in the sample, use Purified Mouse APN/ADP antibody to coat microtiter plate wells, make solid-phase antibody, then add APN/ADP to wells, Combined APN/ADP antibody which With HRP labeled, become antibody - antigen - enzyme-antibody complex, after washing Completely, Add TMB substrate solution, TMB substrate becomes blue color At HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of Mouse APN/ADP in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Materials provided with the kit

1	wash solution	20ml×1bottle	7	Stop Solution	6ml×1 bottle
2	HRP-Conjugate reagent	6ml×1 bottle	8	Standard（240μg/L）	0.5ml×1 bottle
3	Microelisa stripplate	12well×8strips	9	Standard diluent	1.5ml×1bottle
4	Sample diluent	6ml×1 bottle	10	Instruction	1
5	Chromogen Solution A	6ml×1 bottle	11	Closure plate membrane	2
6	Chromogen Solution B	6ml×1 bottle	12	Sealed bags	1

Material to be prepared for the experiment

1、Enzyme Marker
2、Pipettor and Gun Head
3、Plate washer
4、Test tube, centrifugal tube, measuring cylinder, etc.
5、Distilled or deionized water
6、Electrothermal thermostat

Collection of Specimen

Collection of liquid samples

1、Serum: Collected by sterile tube, blood is naturally coagulated at room temperature for 10-20 minutes, and centrifuged at 2-8 C for about 20 minutes (2000-3000 rpm). The supernatant is carefully collected. If precipitation occurs during storage, it should be centrifuged again.

2、PLASMA: EDTA, heparin sodium or sodium citrate should be selected as anticoagulant according to the requirement of the specimen. After 10-20 minutes of mixing, centrifugation at 2-8 C for about 20 minutes (2000-3000 rpm). The supernatant should be carefully collected and centrifuged again if precipitation is formed during storage.

3、Urine: Collected by sterile tube, centrifuged at 2-8 C for about 20 minutes (2000-3000 rpm). The supernatant should be carefully collected and centrifuged again if precipitation is formed during storage.

4、Pleural and ascites: collected by sterile tube, centrifuged at 2-8 C for about 20 minutes (2000-3000 rpm). The supernatant should be carefully collected. If precipitation is formed during preservation, it should be centrifuged again.

5、Cerebrospinal fluid: collected by sterile tube, centrifuged at 2-8 C for about 20 minutes (2000-3000 rpm). The supernatant was carefully collected. If precipitation formed during storage, it should be centrifuged again.

6、Saliva: collected by sterile tube, centrifuged at 2-8 C for about 20 minutes (2000-3000 rpm). The supernatant should be carefully collected. If precipitation forms during storage, it should be centrifuged again.

7、Cell culture supernatant: When detecting secretory components, it is collected by sterile tube. Centrifuge at 2-8 C for about 20 minutes (2000-3000 rpm). Carefully collect the supernatant. If precipitation forms during storage, it should be centrifuged again.

8、Milk: Collect with sterile tube, centrifuge at 2-8 C for about 20 minutes (2000-3000 rpm). Collect supernatant carefully. If precipitation forms during storage, centrifuge again.

9、Honey: collected by sterile tube, centrifuged at 2-8 C for about 20 minutes (2000-3000 rpm). The supernatant should be carefully collected. If precipitation forms during storage, it should be centrifuged again.

10、Whole blood: Collect with sterile tube containing anticoagulant, shake it gently immediately and turn it upside down several times to make the blood and anticoagulant even to prevent blood coagulation.

Collection of solid samples

1、Tissue specimens: After cutting specimens, weigh 1 g of tissue, add 9 mL of PBS at pH 7.2-7.4, and homogenize the specimens adequately by hand or homogenizer. Centrifuge for about 20 minutes (2000-3000 rpm), carefully collect the supernatant. Pack one part to be tested and the rest to be frozen for reserve. If precipitation forms during storage, centrifuge again. For plant tissues, if the homogenate is not good, they are fully ground in liquid nitrogen.

2、Samples of intracellular proteins: Many of the proteins to be tested are not secretory proteins. To detect the proteins in cells, the cells need to be collected, washed, and then the cells are broken by ultrasound, and the supernatant is centrifuged.

(1) Cultured cells

A. Animal cells: The cell suspension was diluted with PBS of ph7.2-7.4 to make the cell concentration reach about 1 million/ml. It is broken by ultrasonic wave to destroy cells and release intracellular components. Centrifugation at 2-8 ℃ for about 20 minutes (2000-3000 RPM), collect the supernatant carefully, and centrifuge again if there is precipitation during storage.

B. Plant cells: The cell suspension was diluted with PBS of PH7.2-7.4, and the cell concentration reached about 1 million/ml. The cell suspension was placed on an ice box. The cell was broken for 2 seconds and cooled for 30 seconds by an ultrasonic breaker, so that the cell could be destroyed and the cell components could be released. Centrifuge at 2-8 C for about 20 minutes (2000-3000 rpm). Carefully collect the supernatant. If precipitation forms during storage, it should be centrifuged again.

(2) Cells of tissue

After cutting specimens, 1 g of tissue was weighed and 9 mL of PBS at pH 7.2-7.4 was added. The specimens were fully homogenized by hand or homogenizer. Centrifuge at 2-8 C for about 20 minutes (2000-3000 rpm). Remove the supernatant and wash the precipitated cells carefully with PBS at pH 7.2-7.4 for three times. Then the cells were broken by the above method.

3、Pharyngeal swab: Add 2 ml of PBS at pH 7.2-7.4, dissolve the head of the swab, shake it well, take out the swab with tweezers and squeeze the liquid dry, centrifuge at 2-8 C for about 20 minutes (2000-3000 rpm). Carefully collect the supernatant. Pack one part to be tested and the rest to be frozen for reserve. If precipitation forms during storage, centrifuge again. If it is to measure secretory proteins, take the supernatant directly to detect, test intracellular proteins, to break up cells.

4、Plant specimens:

A. The fresh weight of each sample should not be less than 50mg;

B. The proportion of tissue homogenate was 10%, that is, 1g tissue was added with 9ml homogenate, PBS was used for homogenization, the concentration was 0.01mol/l, and the pH value was controlled in 7.2-7.4. If the sample weight is adjusted, the corresponding uniform slurry can be adjusted according to the ratio of 1:9;

C. Cut the leaf tissue, put it into a bowl, grind it into powder with liquid nitrogen, and add the amount of homogenized liquid after conversion;

D. The supernatant was centrifuged at 4000-5000 rpm for 15 minutes.

Specimen requirements

1、Extract as soon as possible after Specimen collection,and according to the relevant literature, and should be experiment as soon as possible after the extraction. If it can’t, specimen can be kept in -20 ℃ to preserve, Avoid repeated freeze-thaw cycles.

2、Can’t detect the sample which contain NaN3, because NaN3 inhibits HRP active.

3、The above is a general sample processing method, which can not cover all kinds of samples. For some special samples, it is suggested that the experimenters refer to the published literature and design a reasonable sample processing method by themselves.

Assay procedure

1、Dilute and add sample:Dilute Original density Standard as follow table:

120μg/L	5 Standard	150μl Original density Standard+150μl Standard diluent
60μg/L	4 Standard	150μl 5 Standard+150μl Standard diluent
30μg/L	3 Standard	150μl 4 Standard+150μl Standard diluent
15μg/L	2 Standard	150μl 3 Standard +150μl Standard diluent
7.5μg/L	1 Standard	150μl 2 Standard +150μl Standard diluent

2、Add sample：Set blank wells separately (blank comparison wells don’t add sample and HRP-Conjugate reagent, other each step operation is same). testing sample well. Add 50μl of standard to Microelisa stripplate, add Sample dilution 40μl to testing sample well, then add testing sample 10μl (sample final dilution is 5-fold), add sample to wells , don’t touch the well wall as far as possible, and Gently mix.

3、Incubate: After closing plate with Closure plate membrane ,incubate for 30 min at 37℃.

4、Configurate liquid: 30-fold(or 20-fold) wash solution diluted 30-fold (or 20-fold) with distilled water and reserve.

5、Washing: Uncover Closure plate membrane, discard Liquid, dry by swing, add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by pat.

6、Add enzyme: Add HRP-Conjugate reagent 50μl to each well, except blank well.

7、Incubate: Operation with 3.

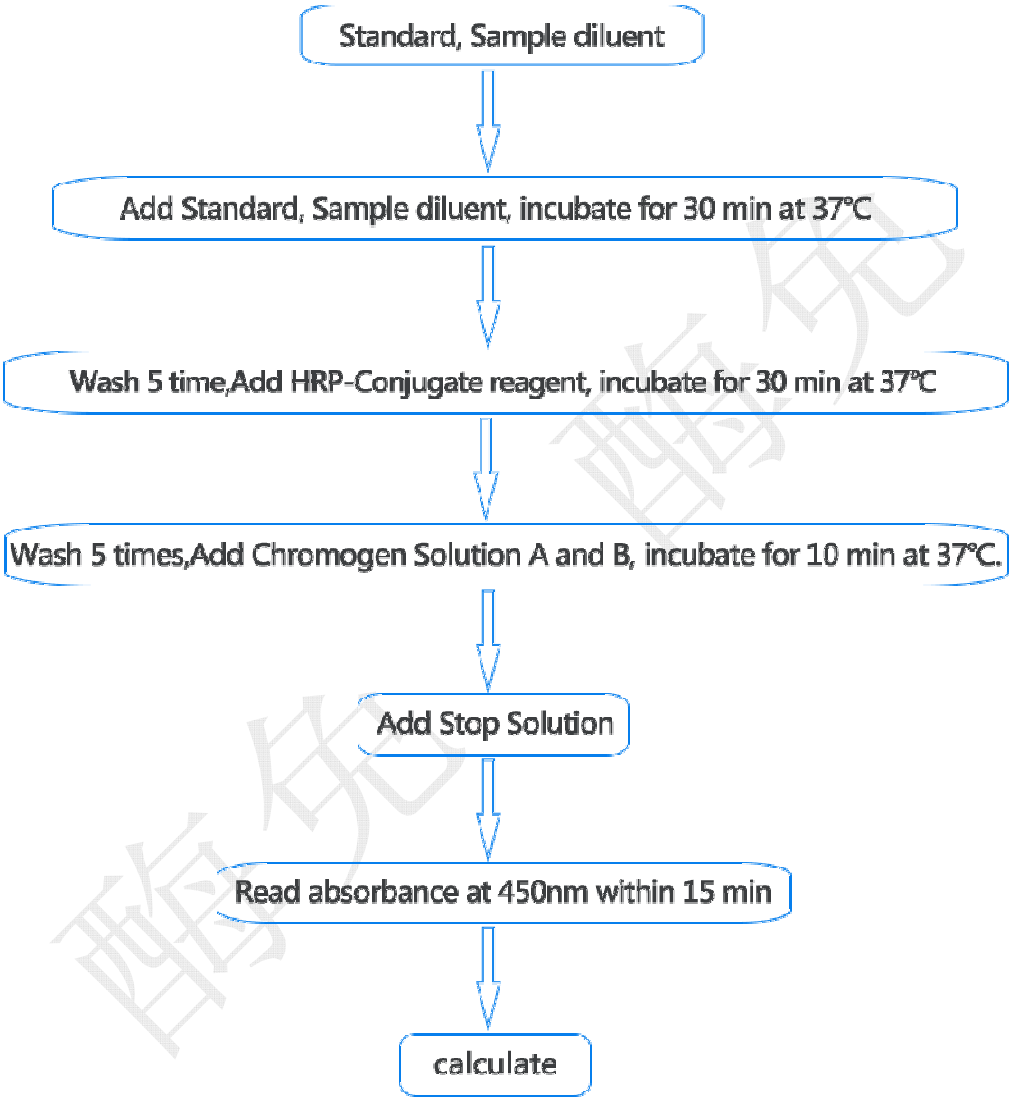
8、Washing: Operation with 5.

9、Color: Add Chromogen Solution A 50μl and Chromogen Solution B 50μl to each well, evade the light preservation for 10 min at 37℃.

10、Stop the reaction:Add Stop Solution50μl to each well, Stop the reaction(the blue color change to yellow color).

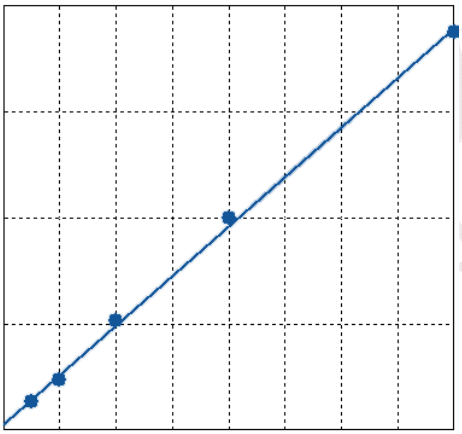
11、Assay: take blank well as zero , Read absorbance at 450nm after Adding Stop Solution and within 15min.

Steps description



Calculate

Take the standard density as the horizontal, the OD value for the vertical ,draw the standard curve on graph paper, Find out the corresponding density according to the sample OD value by the Sample curve, multiplied by the dilution multiple, or calculate the straight line regression equation of the standard curve with the standard density and the OD value ,with the sample OD value in the equation, calculate the sample density, multiplied by the dilution factor, the result is the sample actual density.



This figure is for reference only

performance parameter

1、The correlation coefficient R of sample linear regression and expected concentration is above 0.95.

2、Coefficient of variation within and between batches should be less than 10% and 12% respectively.

Assay range

3μg/L -120μg/L

Storage and validity

1、Storage: 2-8℃

2、Validity: six months

Important notes

1、This kit is used for scientific research, but not for clinical diagnosis.

2、Please use this kit within the validity period of the labeling.

3、Reagents in the kit should not be mixed with other batches of reagents or reagents from other sources.

4、The kit should be removed from the refrigerated environment after 1 hour of room temperature balance. If the enzyme label is not used up after opening, the strip should be stored in a sealed bag.

5、The binding reaction will be affected by the dilution of any standard product, operator, liquid transfer technology, washing technology, incubation temperature, and the change of the storage time of the kit.

6、The kit is designed to remove or reduce some endogenous interference factors in biological samples, not all possible factors have been removed.

7、Concentrated detergent may crystallize. When diluted, it can be heated and dissolved in the water bath without affecting the results of washing.

8、Sampling adders should be used at all steps and their accuracy should be checked regularly to avoid test errors. The best time to add samples is within 5 minutes. If the number of samples is large, it is recommended to use volleyball.

9、Please make standard curves at the same time of each measurement. It's better to make multiple holes. If the content of the substance to be measured in the sample is too high (OD value of the sample is greater than OD value of the first hole of the standard pore), please dilute the sample diluent for a certain number of times (n times) and then determine. When calculating, please multiply the total dilution multiple (*n*5).

10、Sealing film can only be used once to avoid cross-contamination.

11、Substrates should be kept away from light.

12、Strictly follow the instructions. The results of the test must be determined by the reading of the enzyme label.

13、All samples, detergents and all kinds of waste should be treated as infectious substances.

14、The reagent components of different batches should not be mixed.