人钙离子通道抗体（VGCC Ab）酶联免疫

检测试剂盒使用说明书

AE96991Hu

使用前仔细阅读本说明书。本酶联免疫试剂盒是基于双抗体夹心技术原理，来检测人钙离子通道抗体（VGCC Ab），只能用于研究用途，不得用于医学诊断。

用　　途：用于人血清、血浆及相关液体样本中钙离子通道抗体（VGCC Ab）测定。

工作原理

本试剂盒采用的是生物素双抗体夹心酶联免疫吸附法（ELISA）测定样品中人钙离子通道抗体（VGCC Ab）水平。向预先包被了人钙离子通道抗体（VGCC Ab）单克隆抗体的酶标孔中加入人钙离子通道抗体（VGCC Ab），温育；温育后，加入生物素标记的抗 VGCC Ab 抗体。再与链霉亲和素-HRP 结合，形成免疫复合物，再经过温育和洗涤，去除未结合的酶，然后加入底物 A、B，产生蓝色，并在酸的作用下转化成最终的黄色。颜色的深浅与样品中人钙离子通道抗体（VGCC Ab）的浓度呈正相关。

试剂盒组成

试剂盒组成	48 孔配置	96 孔配置	保存
说明书	1 份	1 份	
封板膜	2 片（48）	2 片（96）	
密封袋	1 个	1 个	
酶标包被板	1×48	1×96	2-8℃保存
标准品 160pg/ml	0.5ml×1 瓶	0.5ml×1 瓶	2-8℃保存
标准品稀释液	3ml×1 瓶	6ml×1 瓶	2-8℃保存
链霉亲和素-HRP	3 ml×1 瓶	6 ml×1 瓶	2-8℃保存
生物素标记的抗 VGCC Ab 抗体	0.5ml×1 瓶	1 ml×1 瓶	2-8℃保存
显色剂 A 液	3 ml×1 瓶	6 ml×1 瓶	2-8℃保存
显色剂 B 液	3 ml×1 瓶	6 ml×1 瓶	2-8℃保存
终止液	3ml×1 瓶	6ml×1 瓶	2-8℃保存
浓缩洗涤液	（20ml×20 倍）×1 瓶	（20ml×30 倍）×1 瓶	2-8℃保存

需要而未提供的试剂和器材

1． 37℃恒温箱。

2． 标准规格酶标仪。

3． 精密移液器及一次性吸头

4． 蒸馏水，

5． 一次性试管

6． 吸水纸

注意事项

1．从 2-8℃取出的试剂盒，在开启试剂盒之前要室温平衡至少 30 分钟。酶标包被板开封后如未用完，板条应装入密封袋中保存。按各组分标签温度分开保存。严禁放入冷冻保存。

2．各步加样均应使用移液器，并经常校对其准确性，以避免试验误差。且充分混匀试剂盒里的各种成份及制备的样品。

3．为避免交叉污染，要避免重复使用手中的吸头和封板膜。请勿重复使用已稀释过的标准品、生物素标记抗体、HRP 标记链霉亲和素。

4．底物 B 对光敏感，避免长时间暴露于光下。生物素标记抗体、HRP 标记链霉亲和素使用前请离心处理（5s ）。

5．不同批次的试剂盒组分不能混用。保质期前使用。

6．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.

洗板方法

手工洗板：甩掉酶标板内的液体；在实验台上铺垫几层吸水纸，酶标板朝下用力拍干；将稀释后的洗涤液至少 0.35ml 注入孔内，浸泡 1-2 分钟。洗板 5 次。

自动洗板：甩掉酶标板内的液体；在实验台上铺垫几层吸水纸，酶标板朝下用力拍干；将稀释后的洗涤液至少 0.35ml 注入孔内，间隔 30S 注入吸出。洗板 5 次。

标本要求

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

2．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。若不能马上进行实验，可将标本放于-20℃保存，但应避免反复冻融。

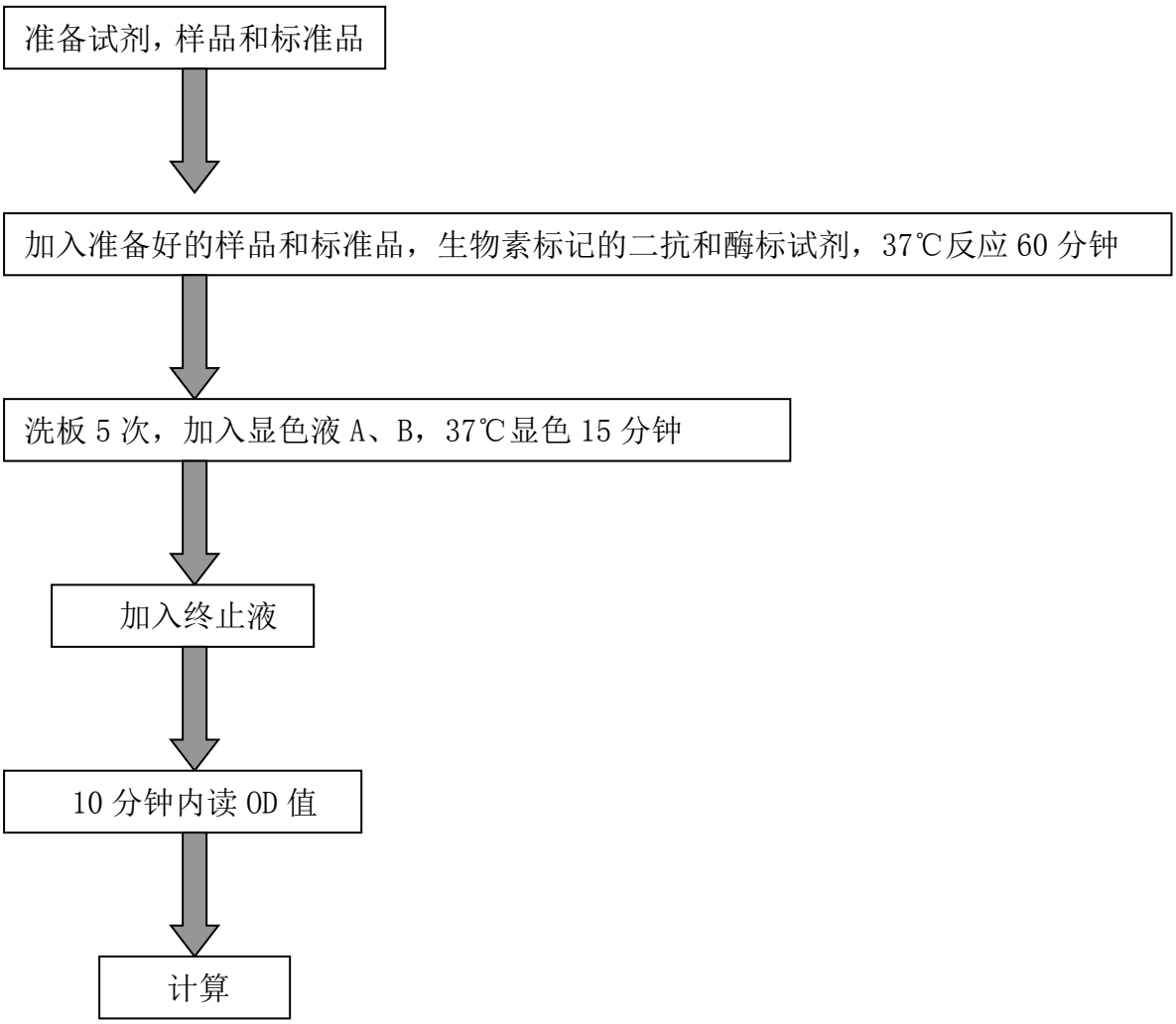
操作程序

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。）

80pg/ml	5 号标准品	120μl 的原倍标准品加入 120μl 标准品稀释液
40pg/ml	4 号标准品	120μl 的 5 号标准品加入 120μl 标准品稀释液
20pg/ml	3 号标准品	120μl 的 4 号标准品加入 120μl 标准品稀释液
10pg/ml	2 号标准品	120μl 的 3 号标准品加入 120μl 标准品稀释液
5pg/ml	1 号标准品	120μl 的 2 号标准品加入 120μl 标准品稀释液

2. 根据待测样品数量加上标准品的数量决定所需的板条数。每个标准品和空白孔建议做复孔。每个样品根据自己的数量来定，能使用复孔的尽量做复孔。
3. 加样：1）空白孔，**空白对照孔不加样品，生物素标记的抗 VGCC Ab 抗体，链霉亲和素-HRP，只加显色剂 A&B 和终止液，其余各步操作相同**；2）标准品孔：加入**标准品 50ul，链霉亲和素-HRP50ul(标准品中已事先整合好生物素抗体，故不加)**；3）待测样品孔：加入**样本 40ul**，然后各加入**抗 VGCC Ab 抗体 10ul、链霉亲和素-HRP50ul**，盖上封板膜，轻轻震荡混匀，37℃温育 60 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. 显色：每孔先加入显色剂 A50ul，再加入显色剂 B50μl，轻轻震荡混匀，37℃避光显色 15 分钟.
7. 终止：每孔加终止液 50μl，终止反应（此时蓝色立转黄色）。
8. 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 10 分钟以内进行。
9. 根据标准品的浓度及对应的 OD 值计算出标准曲线的回归方程，再根据样品的 OD 值在回归方程上计算出对应的样品浓度。建议根据实际检测结果，选择正确的拟合方程，或使用各种应用软件来计算。

操作程序总结：



试剂盒性能：

1.物理性能：各液体组分澄清透明、无沉淀或者絮状物。
2.标准曲线线性：样品线性回归与预期浓度相关系数 R 值为 0.9900 以上。
3.精密度：批内变异系数 CV%小于 9%；批间变异系数 CV%小于 15%。
4.回收率：回收率在 85%-115%之间
5.灵敏度：最低检出剂量小于 1.25pg/ml 。

检测范围：

检测范围： **3pg/ml- 80pg/ml**

保存条件及有效期：

保存：2-8℃。
有效期：6 个月。

Human VGCC Ab ELISA Kit

Instruction

This kit is only for scientific research, and shall not be used as a clinical diagnosis of use.

Purpose

This kit allows for the determination of VGCC Ab concentrations in Human serum, cell culture supernatant, and other biological fluids.

Principle

The kit assay Human VGCC Ab level in the sample， add Human VGCC Ab antibody to microtiter plate wells, after Incubating,add Biotinylated anti–VGCC Ab -antibody , then Combined Streptavidin-HRP, become complex, after Incubating and washing Completely, Add TMB substrate solution,TMB substrate becomes blue color, 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 VGCC Ab in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Materials provided with the kit

Materials provided with the kit	48determinations	96 determinations	Storage
User manual	1	1	
Closure plate membrane	2	2	
Sealed bags	1	1	
Microelisa stripplate	1	1	2-8℃
Standard：160pg/ml	0.5ml×1 bottle	0.5ml×1 bottle	2-8℃
Standard diluent	3ml×1 bottle	6ml×1 bottle	2-8℃
Streptavidin-HRP	3ml×1 bottle	6ml×1 bottle	2-8℃
Biotinylated anti –VGCC Ab -antibody	0.5ml×1 bottle	1ml×1 bottle	2-8℃
Chromogen Solution A	3ml×1 bottle	6ml×1 bottle	2-8℃
Chromogen Solution B	3ml×1 bottle	6ml×1 bottle	2-8℃
Stop Solution	3ml×1 bottle	6ml×1 bottle	2-8℃
wash solution	（20ml×20 fold）	（20ml×30 fold）	2-8℃

	×1bottle	×1bottle	

Materials required but not supplied

1. 37 ℃ incubator
2. Standard microplate reader(450nm)
3. Precision pipettes and Disposable pipette tips.
4. deionized water.
5. Disposable Test tube
6. Absorbent paper

Important notes

1. Reagents stored at 2–8°C should be brought to room temperature and equilibrated for at least 30 minutes before opening the kit. If the coated microplate is not used up after the pouch is opened, return the remaining strips to the sealed resealable bag for storage. Store each component separately at the temperature indicated on its label. Do not freeze.
2. Use a calibrated micropipette for all addition steps, and verify its accuracy regularly to avoid experimental error. Thoroughly mix all kit components and prepared samples before use.
3. To avoid cross-contamination, do not reuse pipette tips or plate sealers. Do not reuse diluted standards, biotin-conjugated antibody, or HRP-conjugated streptavidin.
4. Substrate B is light-sensitive; avoid prolonged exposure to light. Centrifuge the biotin-conjugated antibody and HRP-conjugated streptavidin briefly (5 s) before use.
5. Do not mix components from different lot numbers. Use before the expiration date.
6. Follow the instruction manual strictly. Assay results must be determined based on the microplate reader readings.

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.

Assay procedure

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

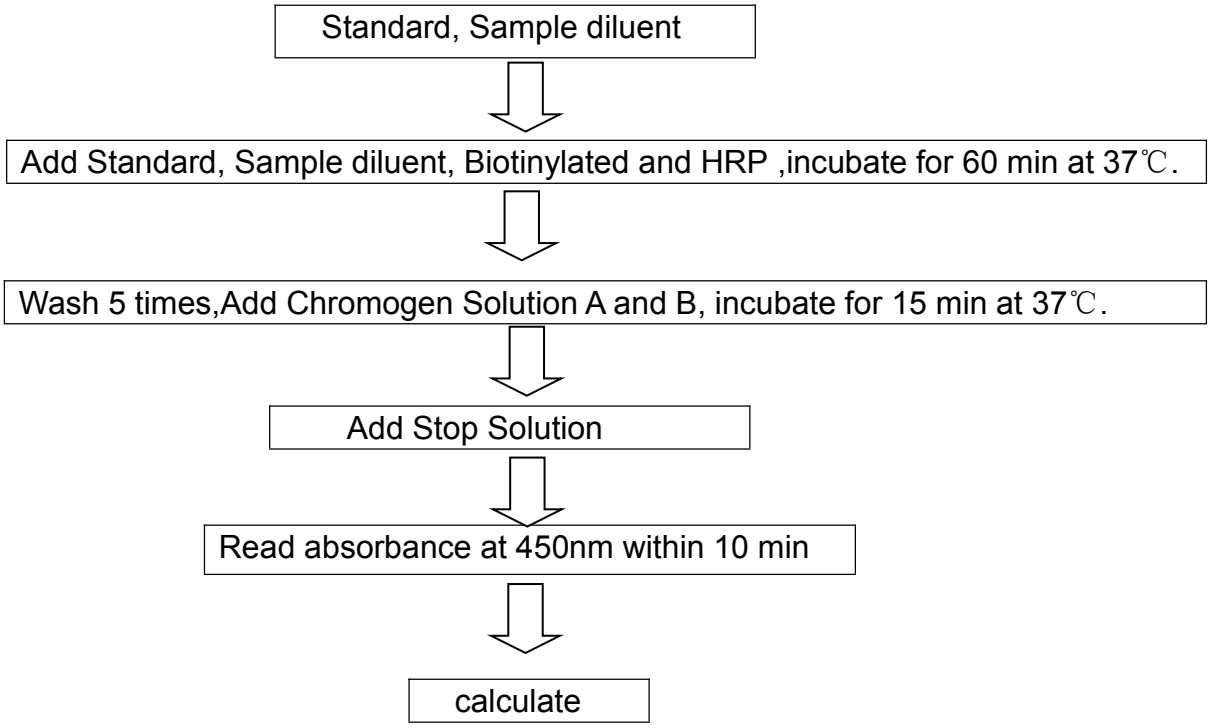
80pg/ml	5 Standard	120μl Original density Standard+120μl Standard diluent
40pg/ml	4 Standard	120μl 5 Standard+120μl Standard diluent
20pg/ml	3 Standard	120μl 4 Standard+120μl Standard diluent
10pg/ml	2 Standard	120μl 3 Standard +120μl Standard diluent
5pg/ml	1 Standard	120μl 2 Standard +120μl Standard diluent

2. according testing Sample numbers to define how many wells need, Standard and blank suggested Do holes.
3. add sample：1) blank wells: (blank comparison wells don't add sample , Biotinylated anti –VGCC Ab -antibody and Streptavidin-HRP ,other each step operation is same); 2) Standard wells: add Standard 50μl and Streptavidin-HRP 50μl; 3) testing Sample wells: add sample 40μl,then add anti –VGCC Ab -antibody 10μl , Streptavidin-HRP 50μl. closing plate with Closure plate membrane ,incubate for 60 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. color：Add Chromogen Solution A 50ul and Chromogen Solution B to each well, evade the light preservation for 15 min at 37℃
7. Stop the reaction：Add Stop Solution50μl to each well, Stop the reaction(the blue color change to yellow color).

8.assay：take blank well as zero , Read absorbance at 450nm after Adding Stop Solution and within 10min.

9. Calculate of result

Steps description



Performance Characteristics

1. Physical properties: All liquid components are clear and transparent, free of precipitate or flocculent matter.
2. Standard curve linearity: The correlation coefficient (R) of the sample linear regression versus the expected concentration is above 0.9900.
3. Precision: Intra-assay coefficient of variation (CV%) is less than 9%; inter-assay CV% is less than 15%.
4. Recovery: Spike recovery falls within 85%–115%.
5. Sensitivity: The minimum detectable dose is less than **1.25pg/ml .**

Assay range

3pg/ml- 80pg/ml

Storage and validity

1. Storage： 2-8℃.
2. validity： six months.