优品生物
UpingBio

(For scientific research use only, not for clinical diagnosis!)

Human Chromogranin A (CHGA)

Quantitative Detection Kit (ELISA)

Instructions for Use Specification:

96T/48T Catalog Number: SYP-H0205

Purpose: Used to detect human chromogranin in serum, plasma, cell culture supernatant and other samples

The concentration of A(CHGA).

Please read the instructions carefully before use. If you have any questions, please contact us via:

Official hotline: 400-999-8863

Technical phone number: 18358180525

Email: UpingBio@163.com

Company website: www.upingbio.com **For specific shelf life, please refer to the outer packaging label of the kit. Please use the kit within the shelf life. When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.**

[Kit performance]

Physical properties: Each liquid component is clear and transparent, with no sediment or floc. Microplate aluminum foil bags should be vacuum packed without damage or leakage.

Calibration curve linearity: The correlation coefficient r value of the calibrator dose-response curve is greater than or equal to 0.9900. Precision: intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%. Sensitivity: The lowest detectable dose is less than 31.25 pg/mL.

Recovery rate: The recovery rate is between 85%-115%.

Sensitivity: This kit recognizes native human chromogranin A (CHGA) and has no crossover with structural analogs.

Stability: Stored at 2℃-8℃, validity period is 6 months.

Detection range: 125 pg/mL – 4000 pg/mL.

【Experimental principle】

This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). In the microwell enzyme plate pre-coated with anti-human chromogranin A (CHGA) antibody (solid-phase antibody), add human chromogranin A (CHGA) calibrator and sample to be tested, and then add biotin labeling After the antibody is incubated and fully washed, HRP-coupled avidin is added. After incubation and sufficient washing, unbound components are removed, and a solid-phase antibody-antigen-biotin is formed on the solid surface of the microplate. Labeled antibody-avidinase sandwich complexes. Add TMB chromogenic solution to produce a blue product. Under the action of the stop solution, it is finally converted into yellow. The absorbance (OD value) is measured on a microplate reader at a wavelength of 450nm. The absorbance (OD value) is related to the human chromaffin granules in the sample to be tested. Protein A (CHGA) concentration is positively correlated. By fitting the calibrator curve, the concentration of human chromogranin A (CHGA) in the sample can be calculated.

[Kit components and storage]

Components		quantity	Main
Calibrator	High Standard	2 vial	Calibrator freeze-
Calibration solution	Reconstitution	2 vial	PBS
Calibrators & Sample Diluents	Standard & Sample Diluent	25mL	PBSTN
coated microplate	Microelisa Stripplate	96T/48T	Pre-coated solid phase
biotin antibody	Bio-Antibody	10mL	biotin antibody
HRP labeled avidin	HRP-Conjugate	10mL	HRP labeled avidin
TMB chromogenic	TMB	10mL	TMB
stop solution	Stop Solution	6mL	acidic solution
20×concentrated	20X Wash Solution	25mL	0.05%Tween20
manual	manual	1 serving	--
Ziplock bag	Ziplock bag	1	--
Self-adhesive	Self-adhesive	4 pieces	--

Note: 1. Before use, please check whether the label and quantity of the reagents in the kit are consistent with the table.

2. The test kit should be stored at 2-8℃. Expired test kits must not be used.

3. If the coated microplate is not used up in a single time, remember to seal it and store it at 2-8℃.

4. The reconstituted calibrator can only be used on the same day.

5. If the components of the kit need to be used again, please ensure that they have not been contaminated since the last use.

试验所需自备试验器材 (不提供，但可协助购买)

1、标准规格酶标仪。

2、自动洗板机。

3、振荡器。

4、系列可调节移液器及吸头，一次检测样品较多时，最好用多通道移液器。

【试剂盒限制性】

1、仅供科研使用，不得用于临床诊断。

2、在试剂盒标示的有效期内使用，过期产品不得使用。

3、跟其他厂家的试剂盒或者组分不能混用。

4、使用试剂盒配套的样品稀释液。

5、如果样本值高于最高校准品浓度值，请将样本适当稀释后，再重新测定。 6、待测样本中存在的人抗鼠等异嗜抗体会干扰检测结果，检测前，请 排除该因素。

7、通过其他方法得到的检测结果，与本试剂盒测定结果不具有直接的可比性。

【注意事项】

1、本试剂盒仅供体外研究使用，不用于临床诊断。

2、试验中请穿着实验服并戴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。

3、严格按照规定的时间和温度进行温育以保证准确结果。所有试剂都 必须在使用前达到室温 20-25℃。使用后立即冷藏保存试剂。

4、洗板不正确可能导致不准确的结果。在加入底物前确保尽量吸干孔 内液体。温育过程中不要让微孔干燥掉。

5、消除板底残留的液体和手指印，否则影响 OD 值。

6、底物显色液应呈无色或很浅的颜色。

7、避免试剂和标本的交叉污染以免造成错误结果。

8、在储存和温育时避免强光直接照射。

9、平衡至室温后再打开密封袋以防水滴凝聚在冷板条上。

10、任何反应试剂不能接触漂白溶剂或漂白溶剂所散发的强烈气体。任何漂白成分都会破坏试剂盒中反应试剂的生物活性。

11、检测使用的酶标仪需要安装能检测 450±10nm 波长的滤光片，

光密度范围在 0-3.5 之间。建议使用时提前 15 分钟预热。

12. Do not mix or replace the reagents in this kit with reagents from other batch numbers or other sources. 13. The EP tubes and suction tips used in the test are single-use and are strictly prohibited from mixing.

14. Do not use expired reagents.

[Preparation and preservation of samples]

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide shall not be used as a preservative. If the sample is not analyzed immediately, it should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

Cell culture supernatant: Centrifuge to remove precipitate, analyze immediately or aliquot and store frozen at -20°C.

Serum: Collect blood in a clean test tube, coagulate at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect serum. Analyze immediately or aliquot and store frozen at -20°C.

Plasma: Anticoagulate with heparin, citrate or EDTA, and centrifuge at 2000 × g for 20 minutes at 2-8°C within 30 minutes of blood drawing. To eliminate the influence of platelets, it is recommended to further centrifuge at 10,000 × g for 10 minutes at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.

Cell lysis medium: For adherent cells, remove the culture medium and replace with PBS, physiological saline or blood-free

Wash with culture medium. Add an appropriate amount of lysis solution and pipet several times to ensure full contact between the lysis solution and cells. Typically after 10 seconds, cells are lysed. For suspended cells, collect the cells by centrifugation and wash them once with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution, pipet with a pipette to disperse the cells, and flick with your fingers to fully lyse the cells. After full lysis, centrifuge at 10000-14000×g for 3-5 minutes and take the supernatant. Analyze immediately or aliquot and store frozen at -20°C.

Urine: Collect in sterile tubes and centrifuge at 2000×g for 20 minutes. Carefully collect the supernatant. If a precipitate forms, centrifuge again.

【Reagent preparation】

1. Before use, all components must be rewarmed for at least 120 minutes to ensure full rewarming to room temperature.

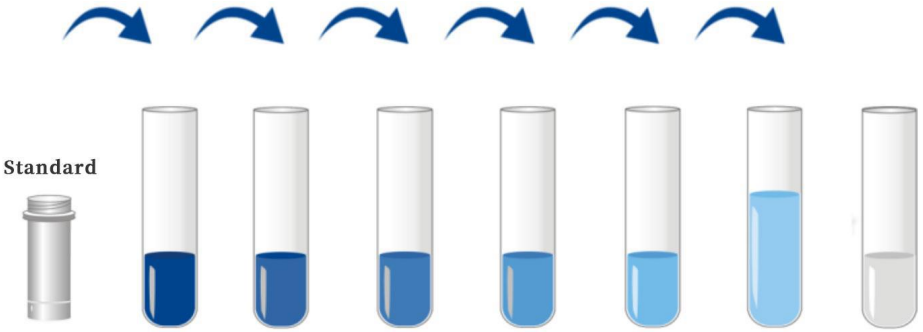
2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating

in a water bath will completely dissolve the crystals. Concentrated detergent and distilled water, dilute 1:20, that is, 1 part of concentrated detergent, add 19 parts of distilled water.

[Calibration dilution method]

Steps for redissolving the calibrator: Redissolve the calibrator with the calibrator reconstituted solution. Add all the liquid in one bottle of the calibrator reconstituted solution to the bottle of calibrator freeze-dried powder. Vortex gently to ensure thorough mixing. , the mother solution concentration of the calibrator after redissolution is 8000 pg/mL , mix thoroughly before dilution.

Dilution steps of the calibrator mother solution: Let the calibrator working solution stand for 1-2 minutes before dilution. Use the calibrator ＆ sample universal diluent to double dilute the calibrator mother solution. The doubling dilution method: take 7 EP tubes, and Add 500 μL of calibrator ＆ sample diluent, pipet 500 μL from the 8000 pg/mL calibrator mother solution into the first EP tube, mix well to prepare a 4000 pg/mL calibrator working solution, and follow this step in sequence. Pipette and mix. As shown on the following page.



8000 pg/mL

Recommended dilution concentration of calibrator: It is recommended to prepare the following concentrations: 4000, 2000, 1000,

500, 250, 125, 0 pg/mL, and used as the calibrator concentration value of the fitting standard curve.

Tip: Add the calibrator ＆ sample universal diluent directly to the last tube as the 0 value. There is no need to draw liquid from the penultimate tube. The diluted calibrator working solution needs to be prepared and used immediately.

【Operating Procedure】

Recommended sample dilution scheme: It is recommended that teachers conduct preliminary experiments to explore the optimal dilution ratio of samples before conducting formal experiments.

All reagents and components should be returned to room temperature first. It is recommended to perform duplicate wells for calibrators, quality control materials and samples.

1. Prepare the working solutions of various components of the kit according to the method described in the previous instructions.

2. Take out the required slats from the aluminum foil bag, seal the remaining slats in a ziplock bag and return it to the refrigerator.

3. Set up the calibrator hole, sample diluent hole, blank hole and sample hole. Add 50 μL of calibrator of different concentrations to each of the calibrator holes. Add 50 μL of sample diluent to the sample diluent hole. Do not add any to the blank hole. Add the test well to the sample hole. Sample 50μL. Except for the blank wells, add 100uL of biotin antibody to each well, cover the reaction plate with a sealing film, and incubate in a 37°C water bath or incubator in the dark for 60 minutes.

4. Uncover the sealing film, discard the liquid, pat dry on absorbent paper, fill each well with washing solution, and let it sit.

1 minute, shake off the washing liquid, pat dry on absorbent paper, repeat this 5 times. If using an automatic plate washer, please wash the plate according to the operating procedures of the plate washer. Adding a soaking program for 30 seconds can improve the detection accuracy. After washing the plate and before adding substrate, pat the reaction plate dry on clean, lint-free paper.

5. Except for the blank wells, add 100uL of HRP-labeled avidin to each well, cover the reaction plate with a sealing film, and incubate in a 37℃ water bath or incubator in the dark for 20 minutes.

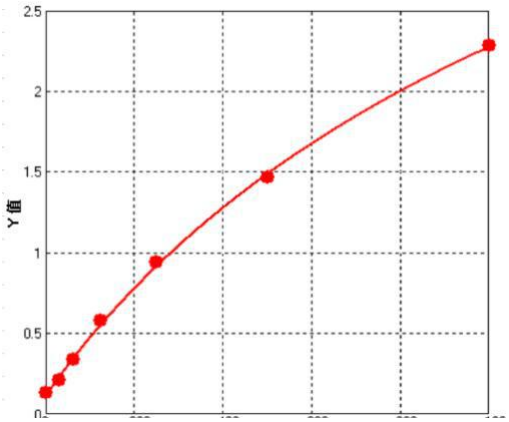
6. Repeat step 4.

7. Add 100μL of TMB chromogenic solution to all wells. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator in the dark for 15 minutes.

8. Add 50 μL of stop solution to all wells, and read the absorbance (OD value) of each well on a 450 nm wavelength microplate reader.

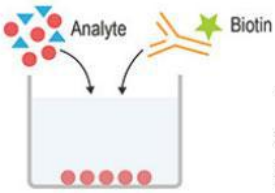
【Result calculation】

1. Use the concentration of the calibrator as the abscissa and the corresponding absorbance (OD value) as the ordinate. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance (OD value) of the sample value), use the equation to calculate the concentration value of the sample. [Calculation using ELISA Calc software] 2. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to obtain the final concentration of the sample.

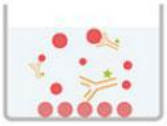


(Schematic diagram, for reference only)

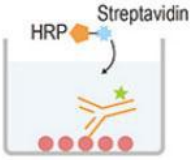
[Operation Summary]



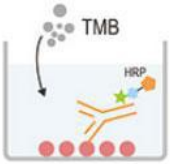
1、反应板孔中加入50uL校准品工作液或样本后,立即每孔加入100uL生物素化抗体工作液，37℃孵育60分钟。



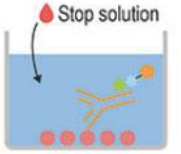
2、弃掉板内液体，洗板5次。



3、每孔加入100uL HRP酶结合物工作液37℃孵育20分钟，弃掉板内液体，洗板5次。



4、每孔加入100uL TMB显色液，37℃孵育 15分钟。



5、每孔加入 50uL 终止液。

6、立即在 450nm波长下读数，处理数据。

【problem analysis】

Problem Description	Possible Causes	Corresponding
Negative and positive control results are unstable	Incorrect liquid	Check pipettes and tips
	Equilibration time is too	Ensure sufficient
	Incomplete washing	Ensure the washing time and number of washes
Very weak or colorless	Incubation time too short	Ensure adequate
	Experimental	Use recommended
	Insufficient reagent	Check the liquid aspiration and addition
	Incorrect dilution	
	Enzyme label inactivation or substrate	Mix enzyme conjugate and substrate and check
Reading value is low	Microplate reader settings are incorrect	Check the wavelength and filter settings on the
		Turn on the microplate
Large coefficient of	Adding fluid incorrectly	Check the filling
High background value	The working	Use the recommended
	Incomplete washing of enzyme plate	Ensure that each step of cleaning is complete; if using an automatic plate washer, please check
	The lotion is	Prepare new lotion
Low sensitivity	Improper storage of ELISA kits	Store relevant reagents according to instructions
	Not terminated before reading	Stop solution should be added to each well

If the experimental results are not good, please take pictures of the color development results in time, save the experimental data, keep the strips used and unused reagents, and then contact our company's technical support to solve the problem for you.

【statement】

1. Due to existing conditions and scientific and technological level, it is not possible to conduct comprehensive identification and analysis of all raw materials. This product may have certain quality and technical risks.

2. This kit removes/reduces some endogenous interfering factors in biological samples during the development process. Not all possible influencing factors have been removed.

3. The final experimental results are closely related to factors such as the effectiveness of the reagents, the relevant operations of the experimenter, and the experimental environment at the time. Our company is only responsible for the kit itself and is not responsible for the sample consumption caused by the

use of the kit. Please use The user should fully consider the possible usage of the sample and reserve sufficient samples before use.

4. In order to achieve good experimental results, please only use the reagents provided in our company's kits, do not mix products from other manufacturers, and operate in strict accordance with the instructions.

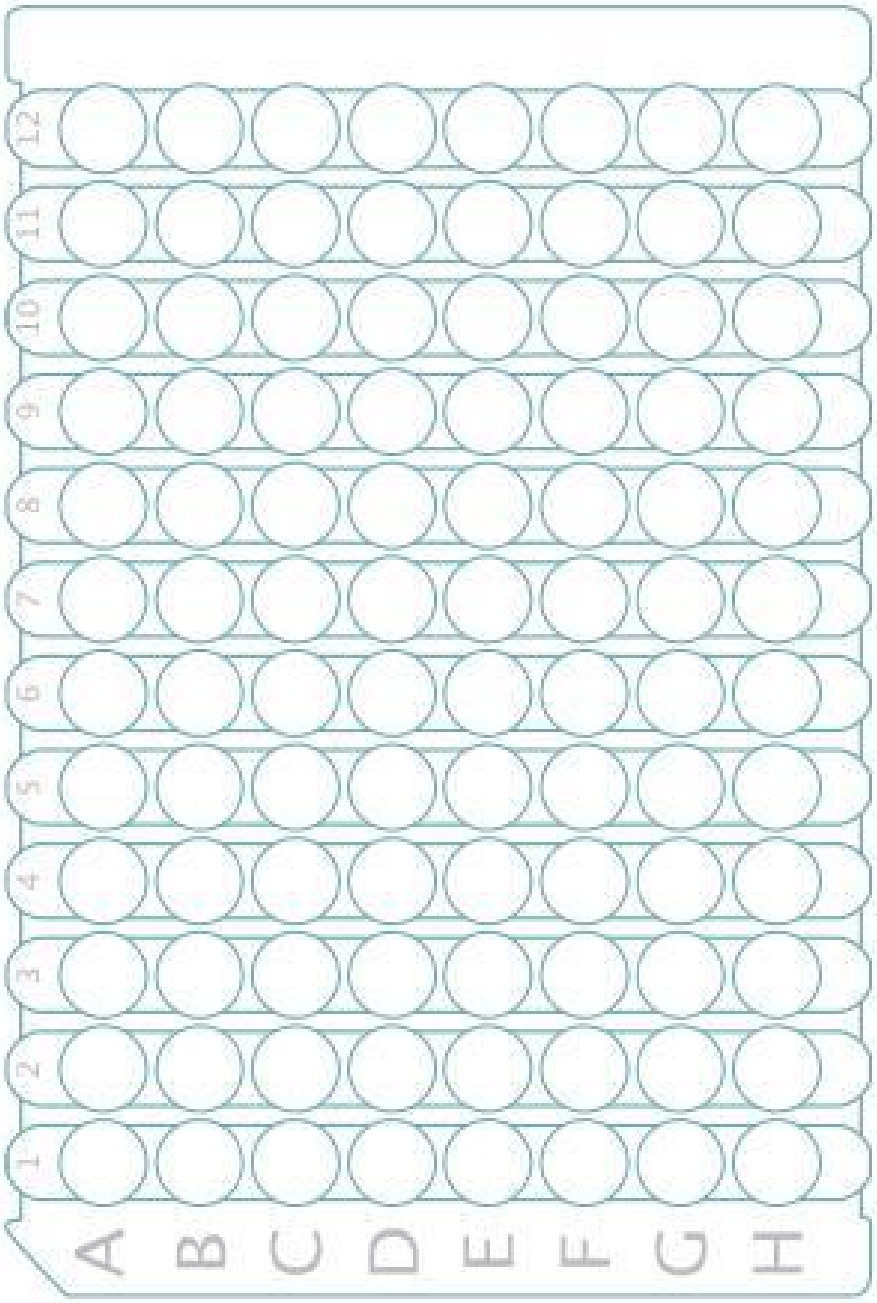
5. Due to incorrect reagent preparation and microplate reader parameter settings during the operation, abnormal results may occur. Please read the instructions carefully and adjust the instrument before the experiment.

6. Even if operated by the same personnel, different results may be obtained in two independent experiments. In order to ensure the reproducibility of the results, it is necessary to control every step of the experimental process.

7. The kits will undergo strict quality inspection before shipment. However, due to factors such as transportation conditions, differences in experimental equipment, etc., user test results may be inconsistent with factory data.

8. This kit has not been compared with similar kits from other manufacturers or products using different methods to detect the same target, so inconsistent test results cannot be ruled out.

9. The kit is for research use only. If it is used for clinical diagnosis or any other purpose, our company will not be responsible for any problems arising therefrom, nor will we assume any legal liability.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【Experimental experience】

【Experimental experience】