优品生物
UpingBio

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

Human aminoacylase 1 (ACY1)

quantitative detection kit (ELISA)

instruction manual Specification:

96T/48T Catalog No.: SYP-H2408

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

1(ACY1) concentration.

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 0.078 ng/ml.

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

Sensitivity: This kit recognizes natural human aminoacylase 1 (ACY1) and has no crossover with structural analogs.

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

Detection range: 0.312 ng/ml – 10 ng/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 aminoacylase 1 (ACY1) antibody (solid-phase antibody), add human aminoacylase 1 (ACY1) 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 aminoacylation in the sample to be tested. The concentration of enzyme 1 (ACY1) is positively correlated. By fitting the calibrator curve, the concentration of human aminoacylase 1 (ACY1) 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.

Prepare your own test equipment required for the test (not provided, but can assist in purchasing) 1.

Standard specification microplate reader.

2. Automatic plate washing machine.

3. Oscillator.

4. A series of adjustable pipettes and tips. When testing a large number of samples at one time, it is best to use a multi-channel pipette.

[Kit limitations]

1. For scientific research use only and not for clinical diagnosis.

2. Use within the validity period marked on the kit. Expired products must not be used.

3. Do not mix with kits or components from other manufacturers.

4. Use the sample diluent provided with the kit.

5. If the sample value is higher than the highest calibrator concentration value, please dilute the sample appropriately and then re-measure. 6. Human anti-mouse

and other heterophilic antibodies present in the sample to be tested will interfere with the test results. Please eliminate this factor before testing.

7. The test results obtained by other methods are not directly comparable to the test results of this kit.

【Precautions】

1. This kit is for in vitro research only and not for clinical diagnosis.

2. Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.

3. Incubate strictly according to the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Store reagents refrigerated immediately after use.

4. Incorrect plate washing may lead to inaccurate results. Be sure to absorb as much liquid as possible from the wells before adding substrate. Do not allow the microwells to dry out during incubation.

5. Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.

6. The substrate chromogenic solution should be colorless or very light in color.

7. Avoid cross-contamination of reagents and specimens to avoid erroneous results.

8. Avoid direct exposure to strong light during storage and incubation.

9. After balancing to room temperature, open the sealed bag to prevent water droplets from condensing on the cold slats.

10. Any reaction reagents must not come into contact with bleaching solvents or strong gases emitted by bleaching solvents. Any bleaching ingredients will destroy the biological activity of the reagents in the kit.

11. The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of 450±10nm.

Optical density ranges from 0-3.5. It is recommended to preheat 15 minutes in advance before use.

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.

【Sample preparation and storage】

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide must 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 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.

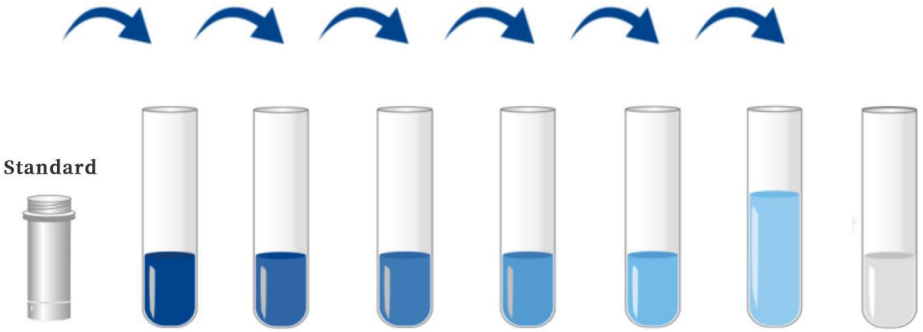
2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating

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 concentration of the mother solution of the calibrator after redissolution is 20 ng/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 calibrator ＆ sample diluent, pipet 500 μL from the 20 ng/ml calibrator mother solution into the first EP tube, mix well to prepare a 10 ng/ml calibrator working solution, and follow this step in sequence. Pipette and mix. As shown on the following page.



20ng/ml

Recommended dilution concentration of calibrator: It is recommended to prepare the following concentrations: 10, 5, 2.5, 1.25, 0.625,

0.312, 0 ng/ml, and used as the calibrator concentration value of the fitted 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 it to the blank hole. Add the sample hole to be tested. 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.

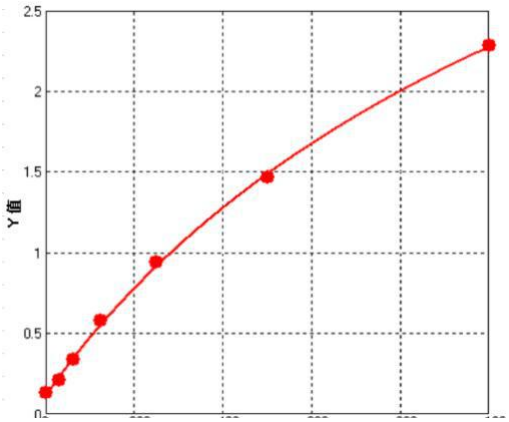
6. Repeat step 4.

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.

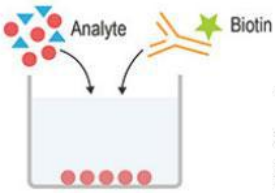
【Result calculation】

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.

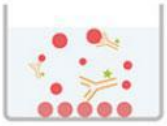


(Schematic diagram, for reference only)

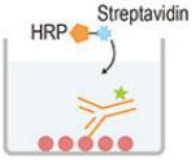
[Operation Summary]



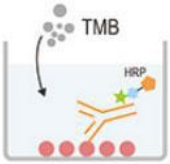
1、反应板孔中加入50uL校准品工作液或样本后,立即每孔加入100uL生物素化抗体工作液，37℃孵育60分钟。



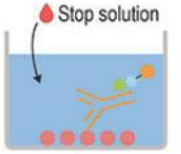
2、弃掉板内液体，洗板5次。



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
	The 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	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	使用推荐的稀释倍数
	酶标板洗涤不完全	保证每步清洗完全；如果用 自动洗板机，请检查所有 的出口是否有堵塞；是否使 用
	洗液有污染	配制新的洗液
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

若实验效果不好，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，然后联系我公司技术支持为您解决问题。

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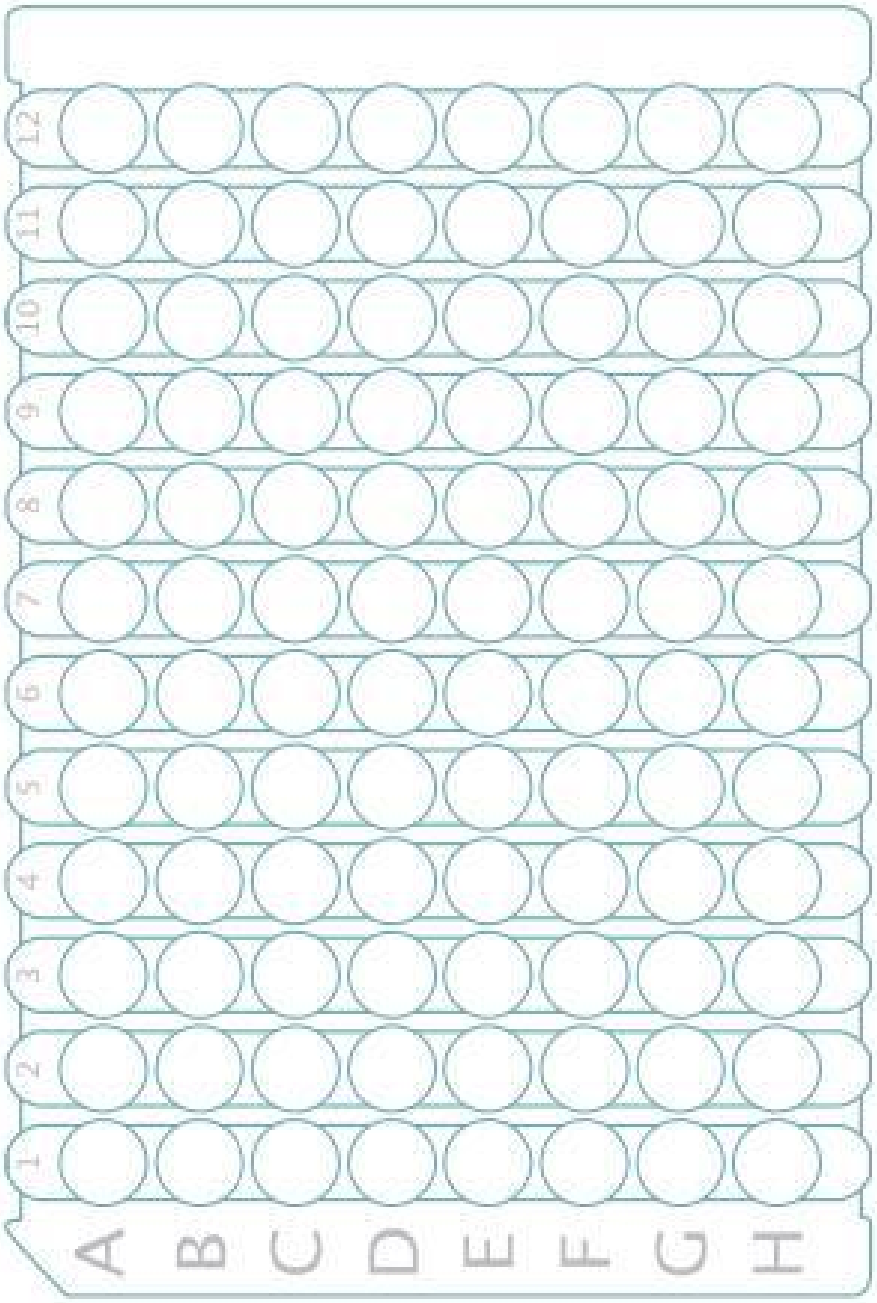
6、即使是相同人员操作也可能在两次独立实验中得到不同的结果，为 保证结果的重现性，需要控制实验过程中每一步的操作。

7、试剂盒发货前会经过严格的质检，然而，因为运输条件、实验设备 差异等等因素影响，用户检测结果可能跟出厂数据不一致。

8、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物的产 品进行对比，所以不排除检测结果不一致的情况。

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】