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

Rat Aquaporin 10 (AQP-10) ELISA Kit

Instructions for Use Product No.: BY-

ER339022 Specifications: 48T/96T Detection

Range: 6.25 pg/ml– 200 pg/ml.

Sensitivity: The lowest detectable dose is less than 1.0 pg/ml.

Precision: intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%.

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

Specificity: This kit recognizes native and recombinant rat aquaporin 10 (AQP-10) and has no crossover with structural analogs. Stability: Stored at 2℃-8℃, validity period is 6 months.

Purpose: Used to detect the concentration of rat aquaporin 10 (AQP-10) in samples such as serum, plasma, cell culture supernatant, and tissue.

Please read the instructions carefully before use. If you have any questions, please contact us through the following methods: Official hotline: 025-5229-8998 Sales department phone: 13914481711 Technical phone: 15950492658 Company website: www.byabscience.cn For the 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.

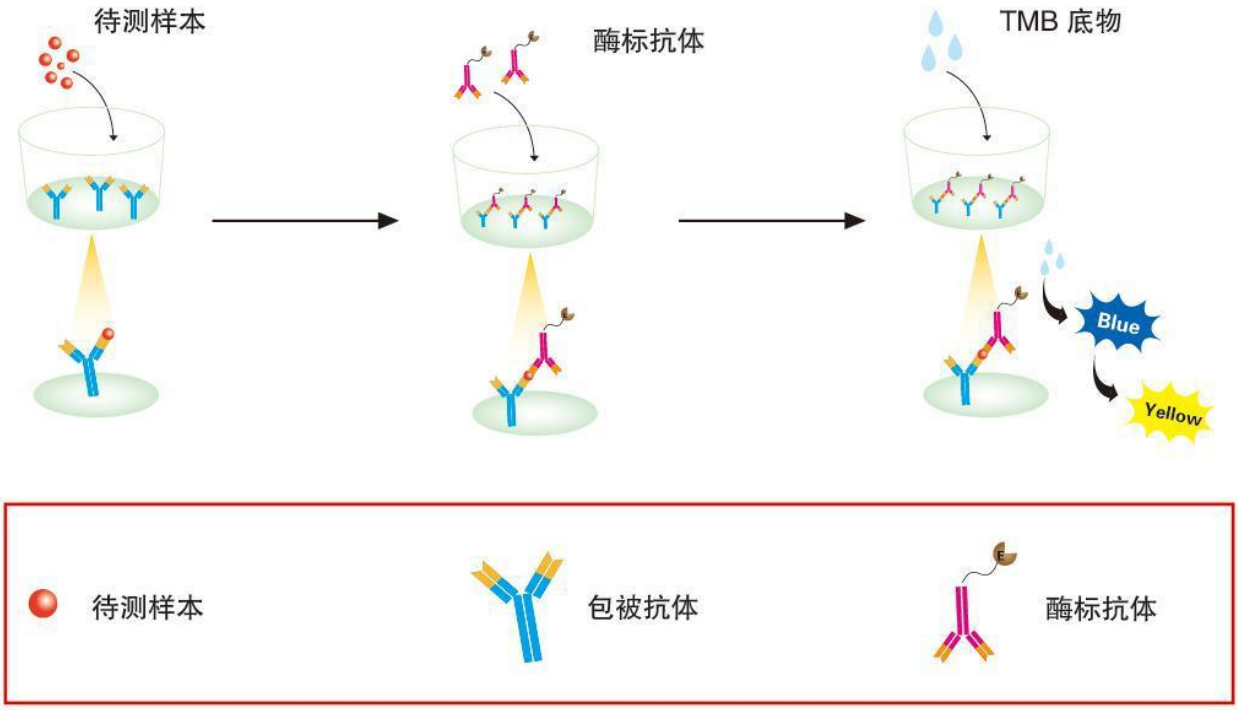
Nanjing BYabscience technology Co.,Ltd

Website: www.byabscience.cn **Official hotline: 025-5229-8998** **Supervision phone number:**

Experimental principle

This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). In the microwell enzyme plate pre-coated with anti-rat aquaporin 10 (AQP-10) antibody (solid-phase antibody), add rat aquaporin 10 (AQP-10) calibrator and sample to be tested, and then Add HRP-labeled anti-rat aquaporin 10 (AQP-10) antibody (enzyme-labeled antibody), and after incubation and sufficient washing, unbound components are removed, and a solid-phase antibody-antigen is formed on the solid surface of the microwell plate. -Sandwich complex of enzyme-labeled antibodies. Add substrates A and B. The substrates are catalyzed by HRP to produce a blue product, which is finally converted to yellow under the action of the stop solution (acidic solution). The absorbance (OD value) was measured at a wavelength of 450 nm using a microplate reader. The absorbance (OD value) was positively correlated with the concentration of rat aquaporin 10 (AQP-10) in the sample to be tested. By fitting the calibrator curve, the concentration of rat aquaporin 10 (AQP-10) in the sample can be calculated.

Experimental schematic diagram



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Kit components and storage: Store unopened kits at 2-8 degrees Celsius. Do not use expired kits.

Components	**48-well configuration**	**96-well configuration**	Store after opening
Pre-coated enzyme plate	48T	96T	**2-8℃14 days**
Standard product	0.3mL*6 tubes	0.3mL*6 tubes	**2-8℃14 days**
sample diluent	3ml	6ml	2-8℃180 days
HRP labeled antibodies	5ml	10ml	**2-8℃14 days**
Chromogenic substrate A	3ml	6ml	2-8℃180 days
Chromogenic substrate B	3ml	6ml	2-8℃180 days
stop solution	3ml	6ml	2-8℃180 days
20×Lotion	15ml	25ml	2-8℃180 days
sealing film	2 sheets	2 sheets	
manual	1 serving	1 serving	
Ziplock bag	1	1	

The concentrations of calibrators are: 200, 100, 50, 25, 12.5, 6.25 pg/ml.

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

2: If the components of the kit need to be used again, please ensure that they have not been contaminated since the last use. 3: If the enzyme plate is not used up in a single time, remember to seal it and store it at 2-8°C.

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

1) Microplate reader capable of detecting absorbance at 450 nm 2) Pipette, pipette tip, and sample addition tank 3) 37°C incubator or water bath 4) Test tubes, centrifuge tubes, measuring cylinders, etc. for preparing

reagents 5) Distilled water or deionized

water Ionized water

6) Vortex shaker, microplate shaker

Notes 1) For scientific research use only, 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. Use the sample diluent provided with the kit.

4) If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately and then re-measure.

5) 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.

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

7) 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.

8) Carry out incubation 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.

9) Improper plate washing can lead to inaccurate results. Make sure to absorb as much liquid as possible from the wells before adding substrate. Do not allow the microwells to dry out during incubation.

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

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

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

13) Avoid direct exposure to strong light during storage and incubation.

14) The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of 450±10nm, and the optical density range is between 0-3.5. It is recommended to preheat 15 minutes in advance before use.

15) The EP tubes and tips used in the test are single-use and are strictly prohibited from mixing.

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 draw. 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 buffer - For adherent cells, remove the culture medium and wash with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution and pipet several times with a gun to fully contact the lysate and cells. Typically after 10 seconds, cells are lysed. For suspended cells, collect the cells by centrifugation and wash them with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution, blow the cells with a gun, and flick them 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.

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。

尿液——用无菌管收集，离心 2000×g 20 分钟。仔细收集上清。如有沉淀形成，应再次离心。

Nanjing BYabscience technology Co.,Ltd

网址：www.byabscience.cn　　官方热线：025-5229-8998　　监督电话：15950492658

试剂准备 1、使用前，所有的组分都要至少复温 60min，确保充分复温到室温。

2、浓缩洗涤液：从冰箱取出的浓缩洗涤液，会有结晶产生，这属于正常现象，水浴加热使结晶 完全溶解。浓缩洗涤液与蒸馏水，按 1:20 稀释，即 1 份的浓缩洗涤液，添加 19 份的蒸馏水。3、底物：底物液 A 和 B，在使用前，按 1:1 体积充分混合，混合后 15 分钟内使用。

操作程序 所有试剂和组分都先恢复到室温，标准品、质控品和样品，建议做复孔。

1、按前面说明书描述的方法，配制好试剂盒各种组分的工作液。

2、从铝箔袋中取出所需板条，剩余的板条用自封袋密封放回冰箱。

3、设置标准品孔、0 值孔、空白孔和样本孔，标准品孔各加不同浓度的标准品 50μL，0 值孔加样本稀释液 50μL，空白孔不加，样本孔加待测样本 50μL。

4、除空白孔外，标准品孔、0 值孔和样本孔，加入辣根过氧化物酶（HRP）标记的检测抗体 100 μL。

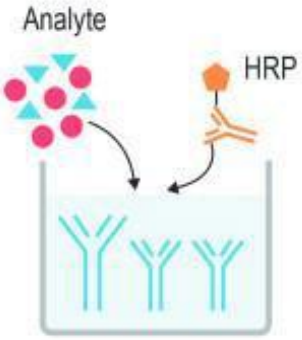
5、用封板膜盖住反应板，37℃水浴锅或恒温箱避光孵育 60min。

6. Uncover the sealing film, discard the liquid, pat dry on absorbent paper, fill each well with washing liquid, let it stand for 20 seconds, shake off the washing liquid, pat dry on absorbent paper, repeat this 5 times. If you use 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. (Tip: In order to obtain ideal experimental results, the residual liquid must be completely removed. After washing the plate, please proceed to the next step immediately and do not let the microplate dry.) 7. Mix substrates A and B at a volume of 1:1 Mix thoroughly and add 100 μL of substrate mixture to all wells.

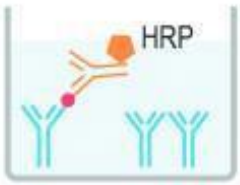
Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator in the dark for 15 minutes.

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

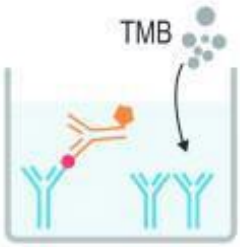
[Operation flow chart]



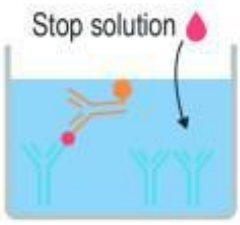
1. 对应板孔中加入50μL标准品工作液或样本后，立即每孔加入100ulHRP酶标抗体工作液，37°C孵育60分钟



2. 弃掉板内液体，洗板5次



3. 每孔加入底物A溶液50ul，底物B溶液50ul



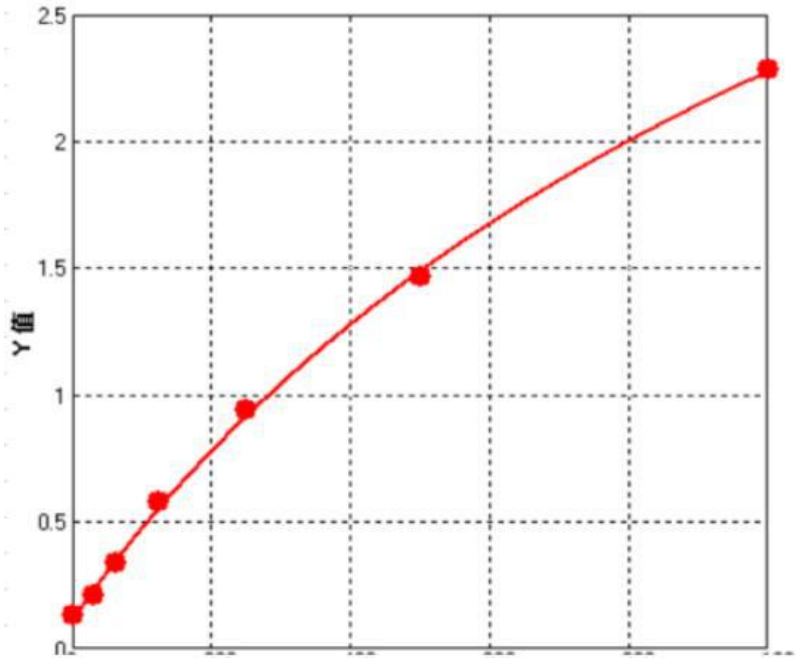
4. 每孔加入50μL终止液

5. 立即在450nm波长下读数，处理数据

Result calculation

9. Use the concentration of the standard substance 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. [Calculate using ELISA Calc software. It is recommended to use four-parameter fitting for the standard curve, but it is not the only fitting method]

10. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to determine the final value of the sample. concentration. Note: Experimenters need to establish a standard curve based on their own experiments. For each test, a standard curve must be established for each enzyme plate. The following curves are for reference only!



(Schematic diagram of the music, for reference only)

[Problem Analysis] If the experimental results are not good, please take pictures of the color development results in time, save the experimental data, keep the used strips and unused reagents, and then contact our company's technical support to solve the problem for you. At the same time, you can also refer to the following information:

[Questions and Answers]

Problem description	Possible reasons	Corresponding countermeasures Corresponding countermeasures
standard curve gradient difference	Incorrect liquid aspiration or	Check pipettes and tips
	平衡时间太短	保证充足的平衡时间
	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	
	酶标记物失活或底物失效	混合酶结合物和底物，通过迅速显色来检 查判断
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	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 试剂盒配备的洗涤液
	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

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