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

Human H1N1 Influenza IgG2b Antibody (H1N1-IgG2b) ELISA Kit Instructions for Use Product No.: BY-EH1102039 Specifications: 48T/96T

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 Contact email: 3224949330@qq.com Company website: www.byabscience.cn For specific shelf life, please see the reagents Box packaging label. 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:**

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

Negative control OD value: less than 0.2.

Positive control OD value: greater than 0.8.

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 human H1N1 influenza IgG2b antibodies (H1N1-IgG2b) and has no crossover with structural analogs.

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

Purpose: For the qualitative detection of human H1N1 influenza IgG2b antibodies (H1N1-IgG2b) in samples such as serum, plasma, cell culture supernatants and tissues.

Shelf life: Stored at 2℃-8℃, valid for 6 months.

Experimental principle

The kit uses an indirect enzyme-linked immunosorbent assay (ELISA). To the microwells pre-coated with human H1N1 influenza IgG2b antibody (H1N1-IgG2b) capture antigen, add the specimen, negative and positive controls in sequence, then add the HRP-labeled detection antibody, incubate and wash thoroughly. The color is developed using the substrate TMB, which is converted into blue under the catalysis of peroxidase and into the final yellow under the action of acid. The depth of the color is positively correlated with the human H1N1 influenza IgG2b antibody (H1N1-IgG2b) in the sample. Use

a microplate reader to measure the absorbance (OD value) at a wavelength of 450 nm to determine negative and positive.

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**
negative control	0.3mL	0.3mL	**2-8℃14 days**
positive control	0.3mL	0.3mL	**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	

Note: 1: Please check whether the label and quantity of the reagents in the kit are consistent with the table before use.

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 oscillator, microplate oscillator

Nanjing BYabscience technology Co.,Ltd

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

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) 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。

9) 洗板不正确可以导致不准确的结果。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。

10) 消除板底残留的液体和手指印，否则影响 OD 值。

11）底物显色液应呈无色或很浅的颜色。

12）避免试剂和标本的交叉污染以免造成错误结果。

13）在储存和温育时避免强光直接照射。

14）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。

15）试验中所用的 EP 管和吸头均为一次性使用，严禁混用。

样品的准备和保存

以下只是列出样品采集和保存的一般指南。所有样本采集保存过程中，不得使用叠氮钠做为防腐剂。样品如果不立即分析，应分装后冷冻保存，且避免反复冻融。

细胞培养上清——离心去除沉淀，立即分析或分装后-20℃冷冻保存。

血清——用干净试管收集血液，室温凝固 30 分钟，离心 2000×g 20 分钟，收集血清。立即 分析或分装后-20℃冷冻保存。

血浆——采用肝素、柠檬酸盐或 EDTA 抗凝，抽血后 30 分钟内在 2-8℃离心 2000×g 20 分钟。为消除血小板的影响，建议在 2-8℃进一步离心 10000×g 10 分钟。立即分析或分装后-20℃冷冻保存。

细胞裂解液——对于贴壁细胞，去除培养液，用 PBS、生理盐水或无血清培养液洗一遍。加入适量裂解液，用枪吹打数下，使裂解液和细胞充分接触。通常 10 秒后，细胞就会被裂解。对于悬浮细胞，离心收集细胞，用 PBS、生理盐水或无血清培养液洗一遍。加入适量裂解液，用枪吹打把细胞吹散，用手指轻弹以充分裂解细胞。充分裂解后，10000—14000×g 离心 3-5 分钟， 取上清。立即分析或分装后-20℃冷冻保存。

Tissue homogenate - rinse the tissue with pre-cooled PBS (0.01M, pH=7.4) to remove residual blood (lysed red blood cells in the homogenate will affect the measurement results), weigh and cut the tissue into pieces. Mix the minced tissue with the corresponding volume of PBS (generally at a weight-to-volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9mL of PBS. The specific volume can be adjusted appropriately according to experimental needs and recorded. It is recommended to add Protease inhibitor) was added to a glass homogenizer and ground thoroughly on ice. In order to further lyse tissue cells, the homogenate can be sonicated or repeatedly frozen and thawed. Finally, centrifuge the homogenate at 5000 × g for 5 to 10 minutes, and take the supernatant for detection.

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 60 minutes to ensure sufficient 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.

Operating procedures: Return all reagents and components to room temperature first. It is recommended to do duplicate holes for standards, quality control materials and samples.

1. Prepare the working solution 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 standard wells and sample wells, and add 50 μL of standards of different concentrations to each standard well;

4. Add 50 μL of the sample to be tested into the sample well; do not add it to the blank well.

5. Except for the blank well, add 100 μL of horseradish peroxidase (HRP)-labeled detection antibody to each well of the standard well and sample well, seal the reaction well with a sealing film, and keep the temperature at 37°C in a water bath or thermostatic oven. Incubate for 60 minutes.

6. Discard the liquid, pat dry on absorbent paper, fill each well with washing solution (350 μL), let it stand for 1 minute, shake off the washing solution, pat dry on absorbent paper, and repeat washing the plate 5 times (you can also use a plate washer to wash it) plate).

7. Add 50 µL each of substrates A and B to each well, and incubate at 37°C in the dark for 15 minutes.

8. Add 50 µL of stop solution to each well, and within 15 minutes, measure the OD value of each well at a wavelength of 450 nm.

[Interpretation of test results]

1. Negative control OD value: less than 0.2.

2. Positive control OD value: greater than 0.8.

3、阳性判断（Cut-Off 值）：阴性对照 OD 值+0.25，样本 OD 值大于阈值，判定为阳性，反之，为阴性。

Nanjing BYabscience technology Co.,Ltd

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

[问题分析] 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。同时您也可以参考以下资料：[问题解答]

问题描述	可能原因	相应对策相应对策
标准曲线梯度差	吸液或加液不准	检查移液器及吸头
	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液

声明

1. 限于现有条件及科学技术水平，尚不能对所有原料进行全面的鉴定分析，本产品可能存在一定的质量技术风险。

2. 本试剂盒在研发过程中去除/降低了生物学样本中的一些内源性干扰因素，并非所有可能影响的因素均已去除。

3. 最终的实验结果与试剂的有效性、实验者的相关操作以及当时的实验环境等因素密切相关，本公司只对试剂盒本身负责，不对因使用试剂盒所造成 的样本消耗负责，请使用者使用前充分考虑到样本可能的使用量，预留充 足的样本。

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 result. 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.

Nanjing BYabscience technology Co.,Ltd

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