



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

Human Anti-Perinuclear Factor

Antibody (APF) ELISA Kit

Instructions for Use Product No.: BY-

EH110866 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.

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**Kit performance Detection range:****2.5 ng/mL– 8 ng/mL.****Sensitivity: The lowest detectable dose is less than 0.1 ng/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 natural and recombinant human anti-perinuclear factor antibodies (APF) and has no crossover with structural analogs.****Stability: Stored at 2°C-8°C, validity period is 6 months.****Purpose: Used to detect the concentration of human anti-perinuclear factor antibody (APF) in samples such as serum, plasma, cell culture supernatant and tissue. Shelf life: Stored at 2°C-8°C, valid for 6 months.****Experimental principle**

The kit uses an indirect enzyme-linked immunosorbent assay (ELISA). To the microwells pre-coated with human anti-perinuclear factor antibody (APF) capture antigen, add the sample to be tested and the standard in sequence, then add the HRP-labeled detection antibody, incubate and wash thoroughly. Adding 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) is measured at a wavelength of 450 nm on a microplate reader. The absorbance (OD value) is positively correlated with the concentration of human anti-perinuclear factor antibody (APF) in the sample to be tested. By fitting the calibrator curve, the concentration of human anti-perinuclear factor antibody (APF) in the sample can be calculated.

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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°C 14 days
Standard product	0.3mL*6 tubes	0.3mL*6 tubes	2-8°C 14 days
sample diluent	3ml	6ml	2-8°C 180 days
HRP labeled antibodies	5ml	10ml	2-8°C 14 days
Chromogenic substrate A	3ml	6ml	2-8°C 180 days
Chromogenic substrate B	3ml	6ml	2-8°C 180 days
stop solution	3ml	6ml	2-8°C 180 days
20×Lotion	15ml	25ml	2-8°C 180 days
sealing film	2 sheets	2 sheets	
manual	1 serving	1 serving	
Ziplock bag	1	1	

The concentrations of calibrators are: 8, 40, 20, 10, 5, 2.5 ng/mL.

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

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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) 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 $450\pm 10\text{nm}$ 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。

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官方热线: 025-5229-8998

监督电话: 15950492658



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 according to the 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 the 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.

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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.

3. Substrate: Substrate solutions A and B, mix thoroughly at a volume of 1:1 before use, and use within 15 minutes after mixing.

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 μ L), 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).

(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. Add 50 μ L each of substrates A and B to each well. Incubate at 37°C in the dark for 15 minutes.

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

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[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. [Calculation using ELISA Calc software]
10. 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. 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)

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[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
	Equilibration time is too short	Ensure sufficient balancing time
	Incomplete washing	Ensure the washing time and number of washes and the amount of liquid added to each hole
Very weak or colorless	Incubation time too short	Ensure adequate incubation time
	The experimental temperature is incorrect	Use recommended experimental temperatures
	Insufficient reagent volume or missing addition	Check the liquid aspiration and addition process to ensure that all reagents are added in sufficient
	Incorrect dilution	
	Enzyme label inactivation or substrate failure	Mix enzyme conjugate and substrate and check by rapid color development
Reading value is low	Microplate reader settings are incorrect	Check the wavelength and filter
		Turn on the microplate reader and preheat it in advance
Large coefficient of variation	Adding fluid incorrectly	Check the filling situation
High background value	The working concentration of the	Use the recommended dilution
	Incomplete washing of enzyme plate	Ensure that each step of cleaning is complete; if using an automatic plate washer, please check whether all outlets are blocked;
	The lotion is contaminated	Prepare fresh lotion
Low sensitivity	Improper storage of ELISA kits	Store relevant reagents according to
	Not terminated before reading	Stop solution should be added to

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