



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

Porcine Neopterin (NP)

ELISA Kit Instructions for

Use 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: 500

pg/mL-8000 pg/mL.

Sensitivity: The lowest detectable dose is less than 10 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 natural and recombinant porcine neopterin (NP) 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 porcine neopterin (NP) 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 enzyme-linked immunoassay method. Biotin-labeled NP is used, and the purified anti-NP antibody is coated on the microwell plate. In the competitive inhibition reaction, a certain amount of solid-phase antibody is used to inhibit the competitive reaction with biotin-labeled NP and non-labeled antigen (calibrator or specimen). The antibody The amount of binding to biotin-labeled NP is inhibited by the amount of non-labeled antigen. The greater the amount of non-labeled antigen, the less the antibody will bind to the biotin-labeled NP, and vice versa. After the reaction is balanced, a solid-phase antibody-biotinylation is formed. NP, and then add enzyme-labeled avidin to form a solid-phase antibody-biotinylated NP-enzyme label-avidin complex. After adding substrate for color development, use a microplate reader to measure the absorbance (OD value) at a wavelength of 450 nm. As the NP concentration increases, the OD value gradually decreases with a good linear

relationship. This kit has the characteristics of high sensitivity, strong specificity, good repeatability, simple and rapid operation, and has reliable detection performance for the reduction or increase of NP in serum.

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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
biotinylated antigen	3ml	6ml	2-8°C 14 days
HRP labeled avidin	3ml	6ml	2-8°C 180 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: 8000, 4000, 2000, 1000, 500 and 0 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

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6) Vortex oscillator and microplate oscillator.

Notes: 1) For scientific research use only,
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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 $450\pm 10\text{nm}$, 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.

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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, normal 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 $5000\times g$ 离心 5~10 分钟, 取上清检测。

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

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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。

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、将预包被板从密封袋中取出，设一个空白对照孔，不加任何液体；每个校准品设 2 孔，每孔加入对应校准品 50 μ l；其余每个检测孔直接加待测血清或质控品 50 μ l。
 - 4、除空白孔外所有孔加入生物素化抗原 50 μ l，混匀，贴上封板膜，置 37 $^{\circ}$ C 温育 60 分钟。
 - 5、手工洗板：弃去孔内液体，洗涤液注满各孔，静置 10 秒甩干，重复 3 次后拍干。洗板机洗板：选择洗涤 3 次程序洗板后拍干。
- （提示：为获得理想的实验结果，必须彻底移除残留液体。洗板完成之后，请立即进行下一步操作，不要让微孔板干燥。）
- 6、每孔加入酶标亲和素 50 μ l（空白对照孔除外），混匀，贴上封板膜，置 37 $^{\circ}$ C 温育 30 分钟。

7、手工洗板：弃去孔内液体，洗涤液注满各孔，静置 10 秒甩干，重复 3 次后拍干。洗板机洗板：选择洗涤 3 次程序洗板后拍干。

8. Add 50 μ l of chromogen A and 50 μ l of chromogen B to each well. After shaking and mixing, place at 37 $^{\circ}$ C to develop color in the dark for 15 minutes. Add 50 μ l of stop solution to each well.

9. Use a microplate reader to read, take the wavelength of 450nm, first use the blank control well to adjust the zero point, and then measure the optical density value (OD value) of each well.

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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
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 washings 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 aspirating and adding process to ensure that all reagents are added in order and in
	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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