Neopterin
ELISA KIT Cat. No. 3410

For Quantitative Determination of Neopterin In Human Serum
For In Vitro Research Use Only

Kit Components (96 tests)	Cat #
Anti-mouse IgG Fc (goat poly coated Strip plate, (96 wells)	3411
Neopterin **Std. A**, 0 nmol/L, 2 ml	3412A
Neopterin **Std. B**, 2 nmol/L, 0.3 ml	3412B
Neopterin **Std. C**, 5 nmol/L, 0.3 ml	3412C
Neopterin **Std. D**, 10 nmol/L, 0.3 ml	3412D
Neopterin **Std. E**, 25 nmol/L, 0.3 ml	3412E
Neopterin **Std. F**, 50.0 nmol/L, 0.3 ml	3412F
Neopterin **Std. G**, 100 nmol/L, 0.3 ml	3412G
Neopterin **Control 1**, 0.3 ml	3413C1
Neopterin **Control 2**, 0.3 ml	3413C2
Stds and Controls prepared human serum base containing <0.05% thimerosal	
Neopterin-HRP **Conjugate**, 6 ml	3414
Neopterin **Antibody**, 12 ml (Green color vial)	3415
Wash buffer (20X), 50 ml	W-20
TMB Substrate Solution, 22 ml	**TMB-22**
Stop Solution, 12 ml	T-12
Complete Instruction Manual	M-3410

Introduction -Neopterin [D-erythro-neopterin] belongs to the group of pteridines. Neopterin as well as other pteridines are derived in vivo from guanosine-triphophate (GTP). Neopterin represents a precursor molecule of biopterin that is an essential cofactor in neurotransmitter synthesis, and it is also involved in variety of oxidation-reduction reactions in the body. The enzyme GTP-cyclohydrolase-I catalyses this reaction in monocytes and macrophages. Neopterin is exclusively released by activated macrophages, and can be determined in serum, plasma and other body fluids. Neopterin biosynthesis is closely associated with activation of the cellular immune system. Higher levels of neopterin were found in patients with viral infections. Antigenic stimulation of human peripheral blood mononuclear cells leads to neopterin release into cell culture medium. Gamma Interferon treatment of macrophages in vitro also increases neopterin levels and generates highly reactive oxygen species (ROS). Neopterin is capable of enhancing ONOO- - as well as Cu(II)-mediated LDL oxidation, whereas 7,8-dihydroneopterin mainly protects LDL from oxidation. Changes in neopterin concentrations in serum or urine can predict complications such as graft rejection in organ transplant recipients. Elevated neopterin levels are found in autoimmune disorders such as

rheumatoid arthritis and systemic lupus erythematosus (SLE). Neopterin levels can be used as prognostic predictors for certain types of malignancies. Measurement of neopterin levels has particular value for monitoring patients infected with HIV. Neopterin is eliminated primarily in the urine, so evaluation of urinary neopterin levels may be useful in assessing activation of the cellular immunity system even in the absence of typical clinical symptoms, since a correlation has been observed with the course of diseases involving cellular immunity activation and urinary levels. Normally samples are not tested for all possible infections. Therefore, the measurement of neopterin in blood donor samples is a useful tool in order to reduce the risk of infections via blood transfusion. Other diagnostic applications for the determination of neopterin are:

· Follow-up of traumatized ICU patients
· Use as prognostic indication in HIV infections and malignant diseases
· Early indication of complications in allograft recipients
· Indication of disease activity in autoimmune diseases
· Diagnosis of viral infections, Differential diagnosis of acute viral and bacterial infections
· Follow-up control of chronic infections and monitoring of immunostimulatory therapy

The determination of neopterin levels in human and animals offers a useful and innovative tool to monitor diseases associated with the activation of cell-mediated immunity.

PRINCIPLE OF THE TEST

Neopterin ELISA kit is based on competitive binding of human Neopterin from serum samples and enzyme-labeled Neopterin to Neopterin specific antibodies immobilized on microtiter plates. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (blue color) is inversely proportional to the amount of Neopterin present in the sample. The reaction is terminated by adding stopping solution (converts blue to yellow). Absorbance is then measured on an ELISA reader at 450 nm and the concentration of Neopterin in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (25-100 ml) and multichannel pipet with disposable plastic tips. Reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

ADI Neopterin ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions. Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), Diluted H2SO4 (1N, stop solution), and Thimerosal (0.02% v/v in standards, conjuagte diluent and HRP-conjugates).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera cannot be immediately assayed, these could be stored at -20oC for up to six months. Avoid repeated zing and thawing of samples. Do not use specimens containing NaN3. Samples appearing turbid should be centrifuged before testing to remove any particulate material. Plasma, urine or other biological fluids not optimized with the kit but may be tested.

REAGENTS PREPARATION

Dilute stock Wash buffer 1:20 with water. Store diluted buffer at 4oC for 1 month.

Dilution of samples: If necessary, diluted sample with normal saline (0.9% NaCl).

All reagents must be at room temperature prior to their use.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8oC until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8oC or stored frozen in small aliquots.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dispense 200-300 ul of wash buffer to all wells. Mix for 5 seconds and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.

1. Label or mark the microtiter well strips to be used on the plate. Dilute the wash buffer with water (1:10).

2. Pipet **25 ul of standards**, controls, and samples into appropriate wells in *duplicate*. For blanks, add 25 ul of zero and 100 ul of diluent only. See worksheet of a typical set-up on page 5.

3. Add **50 ul of ready-to-use enzyme conjugate** into each well**.** Pipet **100 ul of ready-to-use Neopterin antibody** into each well. Mix gently for 5-10 seconds. Cover the plate and incubate in the dark for **90 minutes** at 20-25 oC temp.

4. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 3 times** with 300 ul of 1X wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

5. Add **200 ul TMB substrate solution**. Mix gently for 5-10 seconds. Cover the plate and incubate for **15 mins** in the dark at 20-25 oC temp..

6. Stop the reaction by adding **100 ul of stop solution** to all wells at the same timed intervals as in step 5. Mix gently for 5-10 seconds to have uniform color distribution (blue color turns yellow).

7. Measure the **absorbance at 450 nm** using an ELISA reader within 15 min.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4oC. Do not touch the bottom of the wells.

Limitations

1. All the reagents within the kit are calibrated for the direct determination of Neopterin in human serum. The kit is not calibrated for the determination of Neopterin in plasma, saliva, urine and other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, or improperly stored serum.
3. Any samples or control sera containing azide are not compatible with this kit, as they may lead to false results.
4. Only conjugate diluent may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

CALCULATION OF RESULTS

A. Average the absorbance of all duplicates. Subtract the averaged non-specific binding (NSB) absorbance from the average obtained above. This yields the net absorbance. Divide the net absorbance by the net zero standard absorbance (Bo) to obtain the percent bound (%B/Bo).

B. FORMULA:

$$\%\ B/Bo = \frac{\text{Abs. (sample)} - \text{Abs. (NSB)}}{\text{Abs. (zero standard)} - \text{Abs. (NSB)}} \times 100$$

Abs. = average absorbance of duplicate wells
NSB = non-specific binding (also known as the blank)
Sample = particular serum or standard being calculated
Zero Standard = 0 nmol/L standard or 100% binding wells

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C. Construct a plot of the percent bound (Y-axis) versus the concentration of the neopterin standards ((X-axis) starting with the 0.5 nmol/L point. Either logit-log or semi-logn graph paper may be used. This yields the standard curve.

D. Using the standard curve, determine the neopterin concentration of each sample.

NOTE: Values that bind either higher or lower than the standard curve should not be determined by extrapolation.

XI. COMPUTER ASSISTED DATA REDUCTION

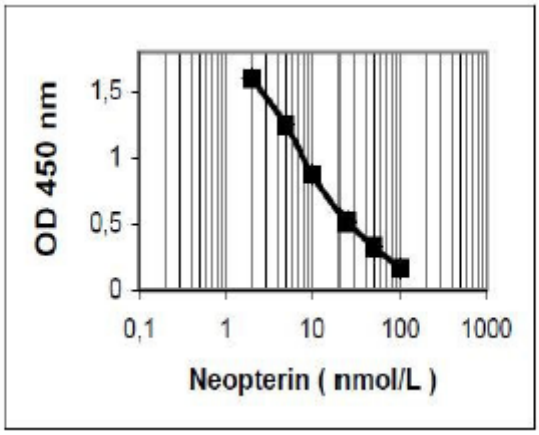
Automated data reduction may be used with a variety of curve fitting algorithms. A smooth curve fit on semi-log or a linear curve fit on logit-log graph paper is recommended. To program your automated data reduction system, please contact your software manufacturer.

Conversion: Neopterin (nmol/L) x 0.253 = ng/mL

WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	Mean A450	%B/Bo
A1, A2	**Std. A** (0 nmol/L)	1, 900	100.0
B1, B2	**Std. B** (2 nmol/L)	1, 600	84.2
C1, C2	**Std. C** (5 nmol/L)	1, 250	65.7
D1, D2	**Std. D** (10 nmol/L)	0, 880	46.3
E1, E2	**Std. E** (25 nmol/L)	0, 520	27.3
F1, F2	**Std. F** (50 nmol/L)	0, 330	17.3
G1, G2	**Std. G** (100 nmol/L)	0, 170	8.9
B3, B4	**Control 1**		
C3, C4	**Control 2**		

NOTE: These data are for demonstration purpose only.



PERFORMANCE CHARACTERISTICS

SENSITIVITY: The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10

replicate analyses) minus 2 SD. Therefore, the sensitivity of the Neopterin ELISA kit is **0.7 nmol/L.**

SPECIFICITY (CROSS REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct Neopterin ELISA kit with Neopterin cross-reacting at 100%. No significant interference was detected at the following concentration. Hemoglobin 35 mg/dL, Bilirubin 2.25 mg/dL, Triglyceride 125 mg/dL

Quality Control

The test results are only valid if the test has been performed following the instructions. All standards and kit controls must be found within the acceptable ranges as stated on the vials. If criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. In case of any deviation the following technical issues should be proven (reagents, protocol, equipments, etc).

EXPECTED NORMAL VALUES

Apparently healthy subjects show the following values:

Normal : <10 nmol/L (0.3-3.0 ng/mL)
Conversion: Neopterin (nmol/L) x 0.253 = ng/mL As with all diagnostic tests, differences in physiological ranges may be encountered from laboratory to laboratory due to patient demographics, laboratory techniques, and population sampling. These ranges should only be used as a guideline. We recommend each laboratory establish its own ranges using a statistically significant number of characterized patient specimens in each diagnostic category.

PERFORMANCE CHARACTERISTICS

Intra-assay precision CV :< 10 % for Neopterin range 2-50 nmol/L
Inter-assay precision CV: <10 % for Neopterin range 2-50 nmol/L

Linearity of Dilutions

Two samples (8 and 12 nmol/L) were serially diluted (1:2-1:8) showed a good correlation (6.8-8 nmol/L).

Recovery

Six samples (0.5-24.0 nmol/L) were spiked with known concn of neopterin and recovery measured (average recovery of 105%).

Antibody Specificity (Cross-reactivity)

Following relevant substances were tested for cross Reactivity: 7,8-Dihydro- Neopterin (3.5%), Monapterin, Biopterin, Dihydro-D-Biopterin, Tetrahydro- Neopterin, and Xanthopterin (<0.05%).

Species reactivity

This kit has been designed and tested for human samples. It may be optimized for other biological fluids. It has not been tested in animals (rat, mouse, etc). Since neopterin is the same in all species, this kit should work in most species s long as the sample cocn is within the range of this kit.

Refs:
Westermann J (2000) Clin. Chem. Lab. Med. 38, 345;
Smith D (2003) Am. Heart J. 146, 69-74

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