

Neopterin ELISA

Enzyme immunoassay for the in-vitro-diagnostic quantitative determination of neopterin in human serum and plasma.



RE59355



5x12x8



2-8°C

EU:



U.S.: *For research use only.
Not for use in diagnostic procedures.*



1. INTENDED USE

Enzyme immunoassay for the *in-vitro-diagnostic* quantitative determination of neopterin in human serum, plasma. This enzyme immunoassay is evaluated for the manual use and especially for the automated use with the DadeBehring BEPIII ELISA processor. Therefore the manual contains to different working procedures. The usage of this assay with other automated systems is possible. However in this case please contact IBL for further advices.

2. SUMMARY AND EXPLANATION

Neopterin biosynthesis is closely associated with activation of the cellular immune system. Increased concentrations of neopterin were reported in patients with viral infections, suggesting that increased values may originate from the immune response of patients directed against virally infected cells. It was shown that antigenic stimulation of human peripheral blood mononuclear cells leads to neopterin release into cell culture medium and that human macrophages produce neopterin *in vitro* when stimulated by interferon gamma. The determination of neopterin levels in human body fluids offers a useful and innovative tool to monitor diseases associated with the activation of cell-mediated immunity. Increasing neopterin levels in various infections precede the clinical manifestation and seroconversion. 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

3. TEST PRINCIPLE

The Neopterin ELISA kit provides material for the quantitative measurement of Neopterin in serum and plasma. The assay procedure follows the basic principle of a competitive ELISA. The peroxidase-conjugated antigen and the unconjugated antigen bind to the rabbit-anti-Neopterin-antibody that itself is bound to an anti-rabbit-antibody (polyclonal, goat) competitively. Both antibodies are immobilized to the wells of microtiter strips. Unbound conjugated and non-conjugated Neopterin is removed by washing. After the substrate reaction the optical density is measured at 450 nm. The amount of peroxidase conjugated antigen bound to the plate and the optical density are inversely proportional to the analyte concentration of the sample. Quantification is possible by comparing the enzymatic activity of the unknown sample with the standard curve.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro* diagnostic use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.

9. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters. The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8°C.

6. SPECIMEN COLLECTION AND STORAGE

Serum, Plasma (EDTA)

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	≤ -20°C (Aliquots)	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.
Stability:	72 h	6 mon	

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
5 x 12 x 8	MTP	Microtiter Plate Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal).
1 x 0.75 mL	ENZCONJ CONC	Enzyme Conjugate, Concentrate (101x) Store protected from light. Contains: Neopterin, conjugated to peroxidase, phosphate buffer, stabilizers.
1 x 6 x 1.5 mL	CAL A-F	Standard A-F 0; 1.35; 4.0; 12.0; 37.0; 111 nmol/L Ready to use. Contains: Neopterin, phosphate buffer, stabilizers.
1 x 2 x 1.5 mL	CONTROL 1+2	Control 1+2 Ready to use. Concentrations / acceptable ranges see QC Certificate.
2 x 100 mL	ASSAYBUF	Assay Buffer Ready to use. Contains: phosphate buffer, BSA, stabilizers.
5 x 10 mL	ASSAYBUF	Assay Buffer Ready to use. Contains: phosphate buffer, BSA, stabilizers.
1 x 100 mL	WASHBUF CONC	Wash Buffer, Concentrate (20x) Contains: Tween, stabilizers.
1 x 70 mL	TMB SUBS	TMB Substrate Solution, Contains: TMB, Buffer, stabilizers.
1 x 90 mL	TMB STOP	TMB Stop Solution Ready to use. 1 M H ₂ SO ₄ .
5 x 1	FOIL	Adhesive Foil (black) For manual use only.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volume: 10; 50; 100; 1000 µL
2. Vortex mixer
3. 8-Channel Micropipettor with reagent reservoirs
4. Wash bottle, automated or semi-automated microtiter plate washing system
5. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
6. Bidistilled or deionised water
7. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

- Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
- Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
- Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
- Use a pipetting scheme to verify an appropriate plate layout.
- Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
- Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
- Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of lyophilized or concentrated components

Amount of enzyme conjugate is calculated for one complete plate.

Dilute/dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
100 mL	Wash Buffer	ad 2000 mL	bidist. water	1:20		2-8°C	1 mon
100 µL	Enzyme Conjugate	with 10 mL	Assay Buffer	1:101	Avoid exposure to light.	2-8°C	7 days

10.2. Dilution of Samples

Samples suspected to contain concentrations higher than the highest standard have to be diluted with Standard A. Avoid in any case the exposure to light. Light leads to a fast degeneration of Neopterin.

11. TEST PROCEDURE

	To perform a valid test run only Standards B-E are necessary. However, Standards A and F can be run in confirmatory assays.
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Recommended Pipetting Scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL B	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat
B	CAL C	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat
C	CAL C	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat
D	CAL D	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat
E	CAL D	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat
F	CAL E	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat
G	CONTROL 1	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat
H	CONTROL 2	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat	Pat

11.1. Procedure for the BEP III

A special program and data base is necessary for valid runs with the DadeBehring ELISA Processor. It can be easily ordered by IBL.

1.	Pipette 100µL of Assay Buffer into all wells.
2.	Pipette 10 µL of each Standard, Control and sample into the respective wells of the Microtiter Plate as mentioned in the pipetting scheme.
3.	Pipette 50 µL of Enzyme Conjugate (1:101) into each well.
4.	Incubate 60 min at RT (18-25°C).
5.	Aspirate supernatant. Wash plate 6 x with 300 µL diluted Wash Buffer .
6.	Pipette 100 µL of TMB Substrate Solution into each well.
7.	Warm up MTP in the 56°C incubator (then incubate plate for 3 min at 37°C).
8.	Incubate 30 ± 5 min at RT (18-25°C).
9.	Pipette 100 µL of TMB Stop Solution into each well.
10.	Measure optical density with a photometer at 450 nm (Reference wavelength: 600-650 nm).



If the assay is operated with an automated system the used reagents should not be discarded; i.e. TMB substrate solution should be filled back into the referring containers and stored at 2-8 °C until reuse.

11.2. General manual procedure

1.	Pipette 100µL of Assay Buffer into all wells.
2.	Pipette 10 µL of each Standard, Control and sample into the respective wells of the Microtiter Plate as mentioned in the pipetting scheme.
3.	Pipette 50 µL of Enzyme Conjugate (1:101) into each well.
4.	Cover plate with black adhesive foil. Incubate 60 min at RT (18-25°C).
5.	Aspirate supernatant. Wash plate 6 x with 300 µL diluted Wash Buffer .
6.	Pipette 100 µL of TMB Substrate Solution into each well.
7.	Incubate 30 ± 5 min at RT (18-25°C).
8.	Pipette 100 µL of TMB Stop Solution into each well.
9.	Measure optical density with a photometer at 450 nm (Reference wavelength: 600-650 nm).

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All kit controls must be found within the acceptable ranges as stated on the vial labels. If the 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: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. For the calculation of the results on the BEP III please use the special product program file available from IBL.

For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The concentration of the samples can be read directly from the standard curve.

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

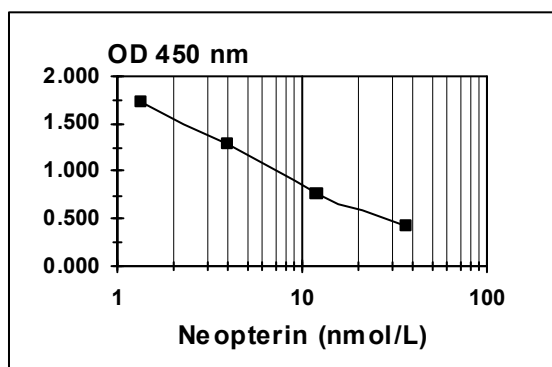
Conversion:

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

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Neopterin (nmol/L)	OD (BEP III)
B	1.35	2.009
C	4.00	1.632 (Mean OD)
D	12.0	1.075 (Mean OD)
E	37.0	0.652

**14. INTERPRETATION OF RESULTS**

Neopterin (Serum)	Interpretation
< 10 nmol/L	normal
> 10 nmol/L	elevated

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

15. EXPECTED VALUES

Apparently healthy subjects show the following values:

Neopterin	Serum	
	nmol/L	ng/mL
	< 10	< 2.5

It is recommended that each laboratory establishes its own range of normal values.

16. LIMITATIONS OF THE PROCEDURE

Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

For cross-reactivities, see PERFORMANCE.

The following blood components do not have a significant effect (+/- 20 % of expected) on the test results up to the below stated concentrations:

Hemoglobin	4.00 mg/mL
Bilirubin	0.50 mg/mL
Triglyceride	30.00 mg/mL

Do not use samples containing sodium azide since these samples lead to erroneous high results.

17. PERFORMANCE

Analytical Specificity (Cross-reactivity)	Substance	Cross Reactivity (%)	Cross-reactivity of other substances tested < 0.05 %	
	Dihydro-Neopterin	3.5		
	Monapterin	0.29		
	Biopterin	0.19		
	Dihydro-Biopterin	0.12		
	Tetrahydro-Neopterin	0.07		
Analytical Sensitivity (Limit of Detection)	0.32 nmol/L	Mean signal (Zero-Standard) - 2SD		
Functional Sensitivity	0.72 nmol/L	CV < 20 %		
Precision	Range (nmol/L)	CV (%)		
	Intra-Assay	1.66 – 44.82	5.5 – 21.7	
	Inter-Assay	1.57 – 39.32	5.4 – 14.3	
Linearity	Range (nmol/L)	Serial dilution up to	Range (%)	
	1.52 – 43.09	1:8	95.3 – 115.0	
Recovery	Mean (%)	Range (%)	% Recovery after spiking	
	99.4	92.1 – 106.7		
Method Comparison versus HPLC	IBL-Assay = 0.51 x HPLC – 1.27		r = 0.99; n = 26	

18. PRODUCT LITERATURE REFERENCES

1. Westermann J, Thiemann F, Gerstner L, Tatzber F, Kozák I, Bertsch T, Krüger C. Evaluation of a New Simple and Rapid Enzyme-Linked Immunosorbent Assay Kit for Neopterin Determination. *Clin Chem Lab Med*, 38 (4): 345-353 (2000)
2. Smith D, Zouridakis, E, Mariani M, Fredericks S, Cole D, Kaski J. Neopterin levels in patients with coronary artery disease are independent of Chlamydia pneumoniae seropositivity. *Am Heart J*, 146 (1): 69-74 (2003)

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di valutazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p>Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.</p>	

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