

Neopterin ELISA

Enzyme immunoassay for the *in-vitro-diagnsotic* quantitative determination of neopterin in human serum, plasma and urine



Manual

Other translations (German, French, Italian, Spanish or Portuguese) of this manual are available on our website www.iqproducts.nl or can be requested at marketing@iqproducts.nl



RE59321



96



2-8 °C



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 and urine.

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

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the basic principle of a competitive ELISA. An unknown amount of antigen in the sample and a fixed amount of enzyme labeled antigen compete for the antibody-binding sites (rabbit-anti-neopterin). Both antigen-antibody complexes bind to the wells of the microtiter strips coated with a goat-anti-rabbit antibody. Unbound antigen is removed by washing. The intensity of the color developed after the substrate incubation is inversely proportional to the amount of antigen in the sample. Results of samples can be determined directly using 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. Do not use specimens containing NaN₃. 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	

Urine

It is possible to use spontaneous as well as 24 h urine. The total volume of urine excreted during a 24 h period should be collected and mixed in a single bottle. Preservation is not necessary. Determine total volume for calculation of results. **Mix and centrifuge samples before use in the assay.**

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
1 x 12x8	MTP	Microtiter Plate Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal).
6 x 0.5 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.
2 x 0.5 mL	CONTROL 1+2	Control 1+2 Ready to use. Concentrations / acceptable ranges see QC Certificate.
1 x 0.1 mL	ENZCONJ CONC	Enzyme Conjugate, Concentrate (201x) Store protected from light. Contains: Neopterin conjugated to peroxidase, phosphate buffer, stabilizers.
1 x 5 mL	ANTISERUM	Neopterin Antiserum Ready to use. Contains: Antiserum (rabbit), phosphate buffer, stabilizers.
1 x 17 mL	TMB SUBS	TMB Substrate Solution, Ready to use. Contains: TMB, Buffer, stabilizers.
1 x 17 mL	TMB STOP	TMB Stop Solution Ready to use. 1 M H ₂ SO ₄ .
1 x 50 mL	WASHBUF CONC	Wash Buffer, Concentrate (20x) Contains: phosphate buffer, Tween, stabilizers.
1 x 18 mL	ASSAYBUF	Assay Buffer Ready to use. Contains: phosphate buffer, BSA, stabilizers.
1 x	FOIL	Adhesive Foil 1 x black


8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipipette Eppendorf or similar devices, < 3% CV). Volume: 10; 50; 100; 1000 μ L
2. Vortex mixer
3. Orbital shaker (500 rpm)
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. 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.
2. 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.
3. 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.
4. Some components contain \leq 250 μ L solution. Take care that the solution is completely on the bottom of the vial before opening.
5. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
6. Use a pipetting scheme to verify an appropriate plate layout.
7. 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.
8. 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.
9. 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

	The contents of the kit for 96 determinations can be divided into 3 separate runs. The volumes stated below are for one run with 4 strips (32 determinations).
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10.1. Preparation of lyophilized or concentrated components

Dilute/dissolve	Component	with	Diluent	Relation	Remarks	Storage	Stability
15 mL	Wash Buffer	285 mL	bidist. water	1:20		2-8 °C	1 mon
25 μ L	Enzyme Conjugate	5 mL	Assay Buffer	1:201	Store protected from light.	2-8 °C	24 h

10.2. Dilution of Samples

Sample	to be diluted	with	Relation	Remarks
Serum	no			Avoid direct sun light.
Urine	generally	Assay Buffer	1:101	e.g. 10 μ L + 1000 μ L. Avoid direct sun light.

Samples containing concentrations higher than the highest standard have to be diluted further.

	<p>Samples from patients treated with ATG (anti-T lymphocyte globulin from rabbit) after transplantation will cause erroneous high results. This effect can be avoided by a pre-incubation of the samples:</p> <p>Pipette 100 µL of serum into a Sarstedt or glass tube and add 200 µL of Assay Buffer. Close tubes (use pierced stopper for glass tubes) and incubate for 10 min in a waterbath at 95 - 100 °C. Vortex and withdraw 10 µL of the gel for the assay. Results have to be multiplied 3-fold.</p>
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11. TEST PROCEDURE

11.1. MANUAL PROCEDURE

1.	Pipette 10 µL of each Standard, Control, serum sample and diluted urine sample into the respective wells of the Microtiter Plate.
2.	Pipette 100 µL of freshly prepared Enzyme Conjugate (1:201) into each well.
3.	Pipette 50 µL of Neopterin Antiserum into each well.
4.	Cover plate with <u>black</u> adhesive foil. Incubate 90 min at RT (18-25 °C) on an orbital shaker (500 rpm) in the dark.
5.	Remove adhesive foil. Discard incubation solution. Wash plate 4 x with 300 µL diluted Wash Buffer . Remove excess solution by tapping the inverted plate on a paper towel.
6.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
7.	Pipette 150 µL of TMB Substrate Solution into each well.
8.	Incubate 10 min at RT (18-25°C) .
9.	Stop the substrate reaction by adding 150 µL of TMB Stop Solution into each well. Briefly mix contents by gently shaking the plate.
10.	Measure optical density with a photometer at 450 nm (Reference-wavelength: 600-650 nm) within 15 min .

IBL-International provides also protocols for commercially available devices e.g. Triturus from Grifols, DSX from Dynex, DS2 from Dynex, Tecan Genesis RSP, BEP3 and BEP2000 from Dade Behring etc. Please contact us if you want to automatize your ELISA. Our application specialists are glad to assist you.

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. A good fit is provided with cubic spline, 4 Parameter Logisitcs or Logit-Log.

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.

Due to the dilution of urine samples the urine values obtained have to be multiplied by the factor 101.

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

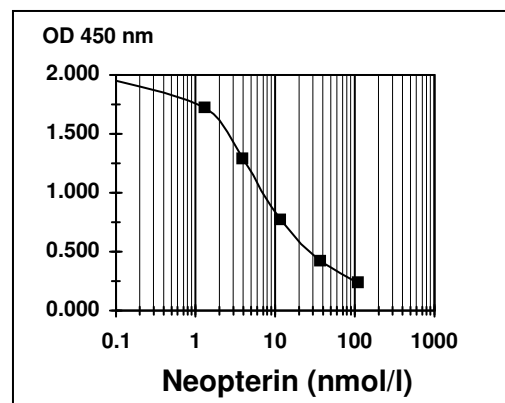
Conversion:

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

Typical Calibration Curve

(Example. Do not use for calculation!)

Standard	Neopterin (nmol/L)	Mean OD	OD/OD _{max}
A	0.00	1.942	100.0
B	1.35	1.713	88.2
C	4.00	1.283	66.1
D	12.0	0.761	39.2
E	37.0	0.412	21.2
F	111	0.237	12.2

**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:

Serum		Urine			
nmol/L	ng/mL	Age	Sex	µmol Neopterin/mol Creatinine	
< 10	< 2.5			Mean	upper limit
		1-4	♂, ♀	267	432
		4-7	♂, ♀	226	405
		7-12	♂, ♀	118	374
		12-15	♂, ♀	171	343
		15-18	♂, ♀	114	320
		18-25	♂	123	195
		26-35	♂	101	182
		36-45	♂	109	176
		46-55	♂	119	197
		>65	♂	133	229
		18-25	♀	128	208
		26-35	♀	124	209
		36-45	♀	140	239
		46-55	♀	147	229
		56-65	♀	156	249
		>65	♀	151	251

(Neopterin Biochemistry – Methods - Clinical Application; H. Wachter et al. (1992), Walter de Gruyter, Berlin - New York)

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	8.33 mg/mL
Bilirubin	0.33 mg/mL
Triglyceride	0.25 mg/mL

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

Samples from patients who were treated with ATG (anti-T lymphocyte globulin from rabbit) after transplantation will cause erroneous high results. This effect can be avoided by a pre-incubation of the samples as described in PRE-TEST SETUP INSTRUCTIONS.

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	0.13		
	Tetrahydro-Neopterin	0.07		
Analytical Sensitivity (Limit of Detection)	0.7 nmol/L	Mean signal (Zero-Standard) - 2SD		
Precision		Range (nmol/L)	CV (%)	
Intra-Assay	Serum	7.7 - 48	3.6 – 6.8	
	Urine	1467 - 6996	7.0 – 8.7	
Inter-Assay	Serum	7.4 - 59	7.6 – 10.3	
	Urine	1212 - 5497	5.8 – 13.2	
Linearity		Range (nmol/L)	Serial dilution up to	Range (%)
	Serum	18.5 – 56.9	1:8	101.0 – 123.2
	Urine	2533 - 5360	1:32	79 - 110
Recovery		Mean (%)	Range (%)	% Recovery after spiking
	Serum	101.2	91 - 112	
	Urine	101.6	91 - 115	
Method Comparison versus HPLC	Serum	IBL-Assay = 0.97 x HPLC - 0.19		r = 0.97; n = 14
	Urine	IBL-Assay = 1.07 x HPLC + 9.2		r = 0.95; n = 104














18. PRODUCT LITERATURE REFERENCES

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19. WARRANTY

Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. IQ Products is not liable for property damage, personal injury, or economic loss caused by the product

20. EXPLANATION OF USED SYMBOLS

	Instructions for use
	Product number
	Amount of tests per package
	For In Vitro Diagnostic use
	Caution, consult accompanying document
	Protect from the light
	Biological risks
	Store at temperature range (°C)
	For Research Use Only
	Batch code
	Use by yyyy-mm-dd
	Manufacturer
	Representative in the European Community

21. CONTACT INFORMATION



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