Product information

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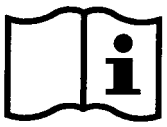

Useŕs Manual

Neopterin ELISA

Enzyme immunoassay for the determination of Neopterin in human serum, plasma and urine

REF **IB79188**

Σ **96 wells**

RUO **For Research Use Only – Not for Use in Diagnostic Procedures**

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1. INTENDED USE

Enzyme immunoassay for the determination of neopterin in human serum, plasma and urine.
For research use only – Not for use in diagnostic procedures.

2. 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 labelled 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.

3. WARNINGS AND PRECAUTIONS

1. For research use only. Not for use in diagnostic procedures.
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-America 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 Demditec-Homepage or upon request directly from IBL-America.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. Avoid contact with Stop solution. It may cause skin irritations and burns.
10. 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. For this reason reagents should be treated as potential biohazards in use and for disposal.

4. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. **Keep away from heat or direct sunlight**. The storage and stability of samples and prepared reagents is stated in the corresponding chapters.
The microtiter strips are stable up to the indicated expiry after the kit is opened. Make sure that the opened bag is tightly closed when stored at 2-8 °C.

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5. SAMPLE 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 sample from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic samples. Do not use samples 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 months	

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 months	

6. MATERIALS SUPPLIED

Quantity	Symbol	Component
12x8	**SORB MT**	Microtiter Plate; Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal).
1 x 8 ml	**ANTISERUM**	Neopterin Antiserum; Ready to use. Contains: Antiserum (rabbit), phosphate buffer, stabilizers.
1 x 13 ml	**ENZ CONJ**	Enzyme Conjugate; Ready to use. Contains: Neopterin, conjugated to peroxidase, phosphate buffer, stabilizers. **Store protected from light.**
6x1.5 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..
2x1.5 ml	**CONTROL 1 & 2**	Control 1+2; Ready to use. Concentrations / acceptable ranges see QC certificate.
1 x 21 ml	**BUF**	Assay Buffer; Ready to use. Contains: phosphate buffer, BSA, stabilizers.
1 x 50 ml	**WASH SOLN 20x**	Wash Buffer Concentrate Concentrate (20x) Contains: Tween, stabilizers.
1 x 19 ml	**SUB TMB**	TMB Substrate Solution; Contains: TMB, Buffer, stabilizers.
1 x 19 ml	**STOP SOLN**	TMB Stop Solution; Ready to use. 1 M H_2SO_4.
1 x	-	Adhesive Foil

7. MATERIALS REQUIRED BUT NOT SUPPLIED

- Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 10; 50; 100; 1000 µL
- Vortex mixer
- Orbital shaker (500 rpm)
- 8-Channel Micropipettor with reagent reservoirs
- Additional assay buffer for urine dilution (can be ordered separately from IBL-America under (cat.-no. DE59321BUF)
- Wash bottle, automated or semi-automated microtiter plate washing system
- Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
- Bidistilled or deionised water
- Paper towels, pipette tips and timer

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8. 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 samples 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 sample. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
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.
Microtiter plate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microtiter plate 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.
Please note that Neopterin is very light sensitive and needs to be protected from light.

9. PRE-TEST SETUP INSTRUCTIONS

Preparation of concentrated components

⚠	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).

Dilute / dissolve	Component	Diluent		Relation	Storage	Stability
15 ml	WASH SOLN 20 x	285 ml	bidist. water	1:20	2-8 °C	1 month

Dilution of Samples

Sample	to be diluted	with	Relation	Remarks
Serum, Plasma	no			**Avoid direct sun light.**
Urine	generally	BUF	1:101	**e.g. 10 µL + 1000 µL** **Avoid direct sun light.**

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

⚠	Samples from subjects 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: Pipette 100 µL of serum into a polystyrene 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 20 µL of the resulting suspension for the assay. Results have to be multiplied 3-fold.

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10. TEST PROCEDURE

Manual Procedure

1. Pipette 20 µL of each Standard, Control, serum, plasma and diluted urine sample into the respective wells of the Microtiter Plate.
2. Pipette 100 µL Enzyme Conjugate into each well.
3. Pipette 50 µL of Neopterin Antiserum into each well.
4. Cover plate with **black 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 300 µL with 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) **in the dark**.
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.

11. 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 comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated on the labels and the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. It is recommended to participate at appropriate quality assessment trials.
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.

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12. 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 Logistics 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:
Based on the molecular weight of Neopterin (MW: 253.2 g/mol) and Creatinine (MW: 113.1 g/mol) a calculation in different units can be made as follows:

Serum/Plasma:

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

Urine:
Usually neopterin in urine is correlated to creatinine (which has to be analyzed by separate method) and expressed in neopterin to creatinine - ratio (UNCR) in µmol neopterin/mol creatinine:

Creatinine	(mg/dL) x 88.4 = (µmol/L)
	(µmol/L) / 1000 = (mmol/L)
	(mmol/L) / 1000 = (mol/L)
Neopterin	(nmol/L) / 1000 = (µmol/L)

13. LIMITATIONS OF THE PROCEDURE

Sample collection and storage have a significant effect on the test results. See SAMPLE 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	5.0 mg/ml
	Bilirubin	2.5 mg/ml
	Triglyceride	45.5 mg/ml

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

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14. PERFORMANCE

<table>
<tr><td rowspan="7">Analytical Specificity (Cross-reactivity)</td><td colspan="2">Substance</td><td colspan="2">Cross Reactivity (%)</td><td rowspan="7">Cross-reactivity of other substances tested < 0.05 %</td></tr>
<tr><td colspan="2">7,8-Dihydro-Neopterin</td><td colspan="2">2.0</td></tr>
<tr><td colspan="2">5,6,7,8-Tetrahydro-Neopterin</td><td colspan="2">< 0.44</td></tr>
<tr><td colspan="2">D-Monapterin</td><td colspan="2">< 0.17</td></tr>
<tr><td colspan="2">L-Monapterin</td><td colspan="2">< 0.03</td></tr>
<tr><td colspan="2">L-Biopterin</td><td colspan="2">< 0.01</td></tr>
<tr><td colspan="2">7,8-Dihydro-L-Biopterin</td><td colspan="2">< 0.03</td></tr>
<tr><td>Analytical Sensitivity (Limit of Detection)</td><td colspan="4">Mean signal (Zero-Standard) - 2SD</td><td>0.7 nmol/L</td></tr>
<tr><td rowspan="5">Precision</td><td colspan="2"></td><td colspan="2">Range (nmol/L)</td><td>CV (%)</td></tr>
<tr><td rowspan="2">Intra-Assay</td><td>Serum</td><td colspan="2">3.1 - 43</td><td>4.3 – 11.7</td></tr>
<tr><td>Urine</td><td colspan="2">932 - 5112</td><td>5.3 – 11.2</td></tr>
<tr><td rowspan="2">Inter-Assay</td><td>Serum</td><td colspan="2">4.67 – 29.98</td><td>8.8 – 13.8</td></tr>
<tr><td>Urine</td><td colspan="2">2616 - 4419</td><td>9.3 – 14.4</td></tr>
<tr><td rowspan="3">Linearity</td><td colspan="2"></td><td>Range (nmol/L)</td><td>Range (%)</td><td>Serial dilution up to</td></tr>
<tr><td colspan="2">Serum</td><td>1.8 – 51.5</td><td>91 – 114</td><td>1:16</td></tr>
<tr><td colspan="2">Urine</td><td>234 - 3622</td><td>87 – 120</td><td>1:8</td></tr>
<tr><td rowspan="3">Recovery</td><td rowspan="3">Recovery after spiking</td><td></td><td colspan="2">Range (%)</td><td>Mean (%)</td></tr>
<tr><td>Serum</td><td colspan="2">81 – 116</td><td>99</td></tr>
<tr><td>Urine</td><td colspan="2"></td><td>94</td></tr>
<tr><td rowspan="2">Method Comparison versus HPLC</td><td>Serum</td><td colspan="3">Assay = 1.18 x HPLC + 0.44</td><td>r = 0.92; n = 111</td></tr>
<tr><td>Urine</td><td colspan="3">Assay = 1.17 x HPLC – 13.52</td><td>r = 0.99; n = 27</td></tr>
</table>

Manufactured for :
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SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore

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