DRG® Neopterin Easy Version ELISA (EIA-4366)

RUO in the USA

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Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 INTENDED USE

Enzyme immunoassay for determination of neopterin in human serum, plasma and urine.
This manual contains two different working procedures. The usage of this assay with other automated systems is possible. However in this case please contact DRG for further advice.

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. 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.
2. In case of severe damage of the kit package please contact 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.
3. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
4. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
5. 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 upon request.
6. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
7. Avoid contact with Stop solution. It may cause skin irritations and burns.
8. 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.

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 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.

5 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.
Stability:	72 h	6 mon	Avoid repeated freeze-thaw cycles.

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.
Stability:	72 h	6 mon	Avoid repeated freeze-thaw cycles.

6 MATERIALS SUPPLIED

Quantity	Symbol	Component
5 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Coated with anti-rabbit IgG (goat, polyclonal).
5 x 5 mL	ANTISERUM	**Neopterin Antiserum** Ready to use. Contains: Antiserum (rabbit), phosphate buffer, stabilizers.
1 x 0.5 mL	ENZCONJ CONC	**Enzyme Conjugate** Concentrate (201x) **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.

Quantity	Symbol	Component
5 x 18 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 90 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_2SO_4.
5 x 1	FOIL	**Adhesive Foil** 5 x black

7 MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volume: 10; 50; 100; 1000 µL
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

8 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. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. 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.
7. 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.

8. 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.

9 PRE-TEST SETUP INSTRUCTIONS

9.1 Preparation of lyophilized or concentrated components

Amounts for Conjugate and Substrate are given for one complete plate.

Dilute/ dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
100 mL	WASHBUF CONC	ad 2000 mL	bidist. water	1:20		2-8°C	1 mon
90 µL	ENZCONJ CONC	with 18 mL	ASSAYBUF	1:201	Store protected from light.	2-8°C	24 h

9.2 Dilution of Samples

Sample	to be diluted	with	Relation	Remarks
Serum	no			Avoid direct sun light.
Urine	generally	ASSAYBUF	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 individuals 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 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.

10 TEST PROCEDURE

10.1 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 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** 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**.

In the following capture is described the procedure for a typical ELISA processor using BEP 2000 from Dade Behring as an example. DRG provides also protocols for other commercially available devices e.g. Triturus from Grifols, DSX from Dynex, DS2 from Dynex, Tecan Genesis RSP, BEP3 from Dade Behring etc. Please contact us if you want to automatize your ELISA. Our application specialists are glad to assist you.

10.2 Procedure for BEP2000, (Dade Behring) for serum and plasma

For valid runs on the Behring ELISA Processor BEP2000 only use the program file and reagent data base that is recommended by DRG. These files can be ordered by DRG easily.

1. Aspirate **110 µL Enzyme Conjugate (1:201)** in one reagent tip (300 µL), and than aspirate additionally to that volume **10 µL** of each **Standard, Control or Sample in the same tip**.
2. Pipette **110 µL** of that mixture (**of Standard, Control or Sample with Enzyme Conjugate**) in the respective wells of the microtiter plate.
3. Pipette **50 µL** of **Antiserum** into each well.
4. **Incubate 90 ± 5min** at **RT (18-25°C)** on an orbital shaker (frequency of 10 Hz; with an amplitude of 4 mm) in the dark.
5. Aspirate supernatant. Wash plate **6 x** with **300 µL** diluted **Wash Buffer**.
6. Pipette **150 µL** of **TMB Substrate Solution** into each well.
7. **Incubate 10 ± 1 min** at **RT (18-25°C)**.
8. Pipette **150 µL** of **TMB Stop Solution** into each well.
9. **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 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. 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.

12 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.

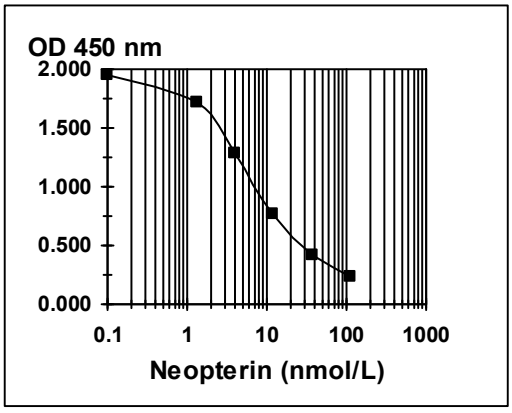
Conversion:

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	0.247	12.2



13 LIMITATIONS OF THE PROCEDURE

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

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 those 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.

14 PRODUCT LITERATURE REFERENCES

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