Human Neopterin ELISA Kit

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

Catalog number: ARG80878

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Neopterin is a low molecular weight molecule belonging to the chemical group known as pteridines. It is synthesised by cellular immune reaction of macrophages and dendritic cells upon stimulation with the cytokine interferon-g and as a consequence released. Neopterin has a higher stability in body fluids which makes the sample handling and measurement easier compared to other cytokines. The low molecular weight, let neopterin molecules rapidly pass the intravasal area, where it is releases in urine after glomerular filtration. The half-life period in human bodies is only affected by renal excretion. So neopterin values reflect the totality of immunological processes for monocytes/macrophages and dendritic cells and can be seen as a general marker of immune activity. This characteristic feature of neopterin to reflect the different interactions of immunocompetent cells is the basis for the extraordinary status of measuring neopterin in immmunological diagnosis. As a non-invasive method, urinary neopterin to creatinine ratio determination is also helpful in monitoring disease progression and the effects of therapies, as well.

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
- diagnosis of tumour diseases
- follow-up control of chronic infections and monitoring of immunostimulatory therapy

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. A specific antibody for rabbit IgG has been pre-coated onto a microtiter plate. Neopterin containing samples or standards, a Neopterin-HRP conjugate and a rabbit antibody for Neopterinn are given into the wells of the microtiter plate. Enzyme labeled and free Neopterin compete for the antibody binding sites.

After incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of Neopterin is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
Neopterin Antiserum	8 ml (ready to use)	4°C
HRP-Neopterin Conjugate	13 ml (ready to use)	4°C
Standards A-F (0, 1.35, 4, 12, 37, 111 nmol/L)	6 X 1.5 ml (ready to use)	4°C
Control Low (5.9 nmol/L; Acc. Range: 3.5 – 8.2 nmol/L)	1.5 ml (ready to use)	4°C
Control High (20.6 nmol/L; Acc. Range: 13.4-27.8 nmol/L)	1.5 ml (ready to use)	4°C
Assay buffer	21 ml (ready to use)	4°C
20X Wash Buffer	50 ml	4°C
TMB substrate	19 ml (ready to use)	4°C (Protect from light)
STOP solution	19 ml (ready to use)	4°C
Adhesive Foil	1	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: 600-650 nm as reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)
- Orbital microplate shaker

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- 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.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 18-25°C) before assay.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the

bottom of tubes.

- The TMB Color developing agent should be colorless and transparent before using.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Incubation time affects results. All wells should be handled in the same order and time sequences
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care.
- Once the test has been started, all steps should be completed without interruption.
- Avoid using reagents from different batches.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot and store samples at ≤ -20°C for up to six month. Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C for up to six month. Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.

Note:

- 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.

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. Assay immediately or aliquot and store samples at ≤ -20°C for up to six month. Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (15 ml 20X wash buffer + 285 ml distilled water). The diluted wash buffer can be stored at 4°C for up to 1 month.
- **Sample**: Urine samples should be diluted 1:101 with Assay buffer. (10 µl Urine + 1000 µl Assay buffer. Avoid direct sun light.) Serum/plasma samples can be assayed directly without dilution. However, if the measuring absorbance of samples is higher than the highest standard, dilute the samples with Assay buffer before assay and assay again.

  Note:

  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 as below:

  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 water bath at 95-100°C. Vortex and withdraw 20 µl of the resulting suspension for the assay. *Results have to be multiplied 3-fold*.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 18-25°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **20 µl** of **Standards, controls and samples** (serum, plasma or **diluted** urine samples) in duplicate into the appropriate wells.
3. Add **100 µl** of **HRP-Neopterin** into each well.
4. Add **50 µl** of **Neopterin antiserum** into each well.
5. Cover wells and incubate for **90 minutes at RT** on an orbital microplate shaker (500 rpm) in the dark.
6. Aspirate each well and wash, repeating the process 3 times for a **total 4 washes**. Wash by filling each well with **1X Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
7. Add **150 µl** of **TMB substrate** to each well. Incubate for **10 minutes at room temperature** in dark.
8. Add **150 µl** of **Stop Solution** to each well. Briefly mix the plate by gently shaking the plate.
9. Read the OD with a microplate reader at **450 nm** immediately. (optional: 600-650 nm as reference wave length) It is recommended read the absorbance within 15 min after adding Stop solution.

Note: For adding of TMB Substrate and Stop Solution, pipetting should be

carried out in the same time intervals. So if it is available, using an 8-channel Micropipettor for adding of TMB Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using a semi-logarithmic or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. 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.

7. Conversion:

Serum/Plasma:

Neopterin (MW: 253.2 g/mol)	(nmol/L) x 0.253 = (ng/ml)
	(ng/ml) / 0.253 = (nmol/L)

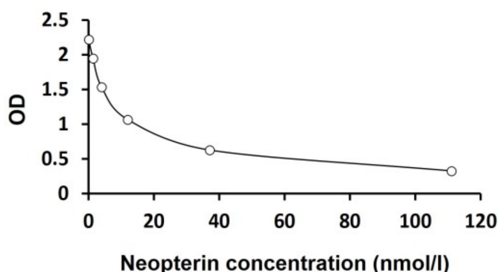
Urine:

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:

Neopterin (MW: 253.2 g/mol)	(nmol/L) / 1000 = (µmol/L)
Creatinine (MW: 113.1 g/mol)	(mg/dL) x 88.4 = (µmol/L)
	(µmol/L) / 1000 = (mmol/L)
	(mmol/L) / 1000 = (mol/L)

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



The O.D. of standard A (0 nmol/L) should >1.000.

QUALITY ASSURANCE

Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of Standard 0 is 0.7 nmol/L.

Specificity

The following substances were tested for cross reactivity of the assay:

Substance	Cross reactivity (%)
7,8-Dihydro-Neopterin	2.0
5,6,7,8-Tetrahydro-Neopterin	< 0.44
D-Monapterin	< 0.17
L-Monapterin	< 0.03
L-Biopterin	< 0.01
7,8-Dihydro-L-Biopterin	< 0.03

Intra-assay and inter-assay precision

The CV value of intra-assay precision was 4.3-11.7% (serum) and 5.3-11.2 (urine).

The CV value of inter-assay precision was 8.8-13.8% (serum) and 9.3-14.4 (urine).

Linearity

Serum: 91-114%

Urine: 87-120%

Recovery

Serum: 81-116%

Urine: 84-117%

Method Comparison versus HPLC:

Serum: Assay=1.18 X HPLC + 0.44 (r=0.92, n=111)

Urine: Assay=1.17 X HPLC- 13.52 (r=0.99, n=27)