Radioimmunoassay (RIA) for the quantitative determination of neopterin in body fluids (Double antibody technique)

Article number: 97R.100 (100 determinations)

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Contents of the Kit

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity for 100 det.</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1 x 11 mL vial</td>
<td>Tracer, Iodine-125-Neopterin (purified by HPLC), red coloured, ready for use, activity: &lt; 110 kBq</td>
</tr>
<tr>
<td>D</td>
<td>1 x 105 mL bottle</td>
<td>Buffer, for the dilution of samples (serum, plasma, urine and other body fluids), ready for use</td>
</tr>
<tr>
<td>L</td>
<td>1 x 11 mL vial</td>
<td>Antiserum, anti-neopterin antibody (sheep) pre-precipitated by anti-sheep IgG antibody (donkey), ready for use. Mix thoroughly before use.</td>
</tr>
<tr>
<td>W</td>
<td>1 x 105 mL bottle</td>
<td>Washing solution, ready for use</td>
</tr>
<tr>
<td>S0 – S6</td>
<td>7 x 0.4 mL vials</td>
<td>Neopterin standards, ready for use, concentrations: 0; 3; 9; 27; 81; 243; 729 nmol/L equivalent to 0; 0.76; 2.3; 6.8; 20.5; 61.5; 184 ng/mL</td>
</tr>
<tr>
<td>K1, K2</td>
<td>2 x 0.4 mL vials</td>
<td>Neopterin control sera K1 and K2 (human serum), ready for use, concentrations see leaflet enclosed</td>
</tr>
</tbody>
</table>

3 sheets of adhesive foil

Instruction Manual B·R·A·H·M·S Neopterin RIA

Date: 16.01.2012
(This version supersedes all earlier instruction manuals.)

Content changes versus previous version

- Insert the address of ALPCO

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Internet
www.thermoscientific.com/brahms
www.thermoscientific.com/procalcitonin
www.thermoscientific.com/kryptor
**Introduction**

The reagents in this kit can be used to assess neopterin levels in serum, plasma (heparin, EDTA, and citrate), cerebrospinal fluid, synovial fluid, ascites, saliva, urine, and other body fluids.

Neopterin (as 7,8-dihydronopterin triphosphate) is an intermediate in the biosynthesis of tetrahydrobiopterin, the cofactor of aromatic hydroxylases. Neopterin is exclusively excreted by activated macrophages and can be determined in serum or other body fluids by immunoassay or HPLC. Elevated neopterin levels can be observed in all diseases with a stimulation of the cellular immune system, e.g. viral diseases, bacterial infections, autoimmune diseases, transplant rejection.

It is present in body fluids partly in hydrogenated forms (dihydro- and tetrahydronopterin) and seemingly in constant ratios. The antiserum in this kit is highly specific for neopterin. As the hydrogenated neopterins can be oxidized to neopterin by iodine, the total amount of neopterin can also be measured by a slight variation of this RIA procedure – a detailed instruction manual is available upon request.

A radioimmunoassay is based on the competition of unlabelled antigen of the samples or standards and radiolabelled antigen (tracer) for the binding sites of the antigen-specific antibody (1. antibody). An antigen-antibody complex is formed. The concentration of the tracer (relative excess as compared to the first antibody) and the concentration of the first antibody are constant in all the tubes within one assay. Consequently, the only variable parameter of the system is the concentration of unlabelled antigen (standards or samples). As the concentration of unlabelled antigen in the sample increases, the binding of the competing tracer molecules to the first antibody is more and more inhibited, i.e. the radioactivity of the antigen-antibody complex is reversely proportional to the concentrations of unlabelled antigen in the sample.

After the reaction is completed the antigen-antibody complex (bound fraction B) is separated from the free antigen fraction (free tracer molecules included) by centrifugation. Finally the radioactivity of the precipitate (bound fraction B) is measured. With the aid of the standards analyzed in parallel (known concentrations of unlabelled antigen) a radioactivity-concentration-profile (standard curve) is constructed. The radioactivity of the samples is then used to determine the corresponding antigen concentrations.

**Quality control:** National quality assurance guidelines for quantitative tests in the medical laboratory (current version) must be complied with. For instance, test accuracy and precision can be monitored by means of laboratory in-house and/or commercially available control materials. If unacceptable control values are obtained, proceed as outlined in standard laboratory diagnostic procedures to determine the cause and implement corrective measures.

**Bibliography**

Test Procedure

Incubation Scheme

1. Number Test tubes (a, b) T S0 – S6 7 etc.
2. Pipette standards µL – 20 (50) –
   samples
3. Pipette tracer µL 100 – 100
4. Pipette antiserum* µL – 100
5. Incubate – 1 h at room temperature under exclusion of light
6. Pipette washing solution mL – 1 1
   Centrifuge 10 min at 2000 x g
7. Decant (or aspirate) – Decant (or aspirate)
8. Measure radioactivity (Counting time: 1 min)

Calculation of results * mix thoroughly before use

Note Usually, the assay should be run with a sample volume of 20 µL for standards and patient samples (20 µL-
version). If a differentiation of neopterin levels below a concentration of 10 nmol/L has to be done (e.g. in
normal sera, CSF etc.), we recommend the use of the 50 µL-version (sample volume of 50 µL for standards
and patient samples).

Specimen Handling

Neopterin and its hydrogenated derivatives are sensitive to light. Therefore samples, standards, and tracer are to be protected from light and kept cool. Direct sunlight has to be avoided in any case.

If samples cannot be analyzed immediately, they can be stored protected from light for 3 days at 2…8°C or for longer periods of time at – 20 °C. Repeated freezing and thawing must be avoided.

Notes on Test Execution

Do not use any reagents that have exceeded the expiration date printed on the label.

The individual components of the kit are perfectly attuned to each other. If components from different batches are exchanged or mixed, B·R·A·H·M·S GmbH can assume no liability for the accuracy of results.

In large test series, reagents of the same batch designation are pooled.

The indicated sequence of steps must be followed.

Patient samples with a concentration above the measuring range are to be rated as “> highest standard”. The result must not be extrapolated. The patient sample in question should be diluted and restered. Further information can be obtained from B·R·A·H·M·S GmbH customer service.

Stability and Storage Conditions

Store all reagents at 2 to 8 °C in their original shipping containers until directly prior to use. Observe the expiry dates specified on the main container and the vial labels. The shelf life of the kit is determined by the shelf life of the tracer.

Test Procedure

Let all reagents and the samples reach room temperature before starting the assay. Agitate standards, samples, and the antibody suspension gently before use (avoid foam formation).

Prepare washing solution: dilute 10.5 mL concentrate with distilled water to yield 500 mL. We strongly recommend to contact the manufacturer or distributor before using other washing solutions.

Note Normal indoor light during the test procedure has generally no effect; however, incubation should be carried out with protection from light.

1. Number the tubes serially (a, b for duplicate), label for total radioactivity tubes: T a, b.
2. Into the tubes S0 a, b ... S6 a, b pipette 20 (50) µL of neopterin standards with increasing concentrations and into the tubes T a, b etc. 20 (50) µL of each sample.
3. Into all tubes pipette 100 µL of tracer (iodine-125-neopterin). The tubes T a, b are now kept separately until the radioactivity is measured (cf. point 8).
4. Into all tubes (except T a, b) pipette 100 µL of preprecipitated antiserum (preferably by a repeating pipette). Agitate the tubes.

Note Mix the suspension thoroughly before use to ensure homogeneity.

5. Cover the tubes with adhesive foil and incubate them for at least 1 hour at room temperature.
6. Into all tubes (except T a, b) pipette 1 mL of washing solution. Agitate the tubes and centrifuge them – if possible all at once – for 10 minutes at a minimum of 2000 x g.

Note If the tubes cannot be centrifuged all at once, add the washing solution only to those tubes which are to be centrifuged and leave the remaining tubes without washing solution until they will be centrifuged.

7. Decant the supernatant from all tubes (except T a, b) and remove any droplets by dabbing on blotting paper. When aspirating care must be taken not to contact the pellet. The device has to be adjusted to a constant distance of about 5 mm from the bottom of the tube. Using this procedure the supernatant will be removed without removing any parts of the pellet.

Note Conical tubes should always be used.

8. Measure the radioactivity of each tube including T a, b. Recommended counting time: 1 minute.

Carefully follow the manufacturer’s instructions. Improper handling of the reagents may falsify the test results. B·R·A·H·M·S GmbH is not liable for faulty test results arising from improper storage, use or handling.

Additionally required
- micropipettes (20, 50, 100 µL)
- dispenser (1 mL)
- sample mixer (vortex or equivalent)
- centrifuge with multispace inserts (minimum 2000 x g)
- gamma counter
Calculation of Results

For computer-aided evaluation, an evaluation programme (spline/unsmoothed) must be selected that suits the specific combination of processor and measuring equipment used.

When calculating the results without assistance of a computer, the mean count rate of each sample (B) should be related to the mean count rate of the zero standard (B₀). The results should be expressed as B/B₀ (%).

In case of the zero standard B₀ = 100 %.

Using semilogarithmic paper the mean percent value of each standard (ordinate, linear) is plotted against the corresponding neopterin concentration (abscissa, logarithmic), in order to obtain a standard curve. The mean percent values B/B₀ of the serum samples are then used to determine the corresponding neopterin concentrations in nmol neopterin/L (e.g. ng/mL).

**Conversion factor: nmol/L x 0.253 = ng/mL**

To check the binding capacity of the whole assay system B₀/T is calculated:

\[
\frac{B₀}{T} \times 100 \%
\]

Under the assay conditions described B₀/T should be in the range of 55 – 85 %.

For technical support, please contact the customer service of B·R·A·H·M·S GmbH or the appropriate distribution partner / sales representative.

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**Example (20 µL-version)**

<table>
<thead>
<tr>
<th>Test tubes</th>
<th>cpm (a)</th>
<th>cpm (b)</th>
<th>cpm (mean)</th>
<th>B/B₀ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total activity</td>
<td>24 865</td>
<td>24 840</td>
<td>24 852</td>
<td></td>
</tr>
<tr>
<td>Zero standard 0</td>
<td>17 248</td>
<td>17 410</td>
<td>17 329</td>
<td>100</td>
</tr>
<tr>
<td>Standard 1 (3 nmol/L)</td>
<td>15 719</td>
<td>15 882</td>
<td>15 800</td>
<td>91.2</td>
</tr>
<tr>
<td>Standard 2 (9 nmol/L)</td>
<td>13 179</td>
<td>12 949</td>
<td>13 064</td>
<td>75.4</td>
</tr>
<tr>
<td>Standard 3 (27 nmol/L)</td>
<td>8 822</td>
<td>8 778</td>
<td>8 800</td>
<td>50.8</td>
</tr>
<tr>
<td>Standard 4 (81 nmol/L)</td>
<td>4 043</td>
<td>3 949</td>
<td>3 991</td>
<td>23.0</td>
</tr>
<tr>
<td>Standard 5 (243 nmol/L)</td>
<td>1 919</td>
<td>1 853</td>
<td>1 891</td>
<td>10.9</td>
</tr>
<tr>
<td>Standard 6 (729 nmol/L)</td>
<td>867</td>
<td>851</td>
<td>859</td>
<td>5.0</td>
</tr>
<tr>
<td>Patient sample 7</td>
<td>14 234</td>
<td>13 880</td>
<td>14 057</td>
<td>81.1</td>
</tr>
</tbody>
</table>

**Standard Curve (20 µL and 50 µL version)**

20 µL version

50 % intercept: approx. 27 nmol/L (6.8 ng/mL)

50 µL version

50 % intercept: approx. 11 nmol/L (2.8 ng/mL)

**Interferences**

<table>
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<tr>
<th>Factor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>no significant effect up to 200 mg/dL</td>
</tr>
</tbody>
</table>

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**Important Notes**

This kit contains materials of human origin (e.g. human serum). These materials have been screened for HBsAg, HIV I/II antibodies, and HCV antibodies; all tests were negative. However, the reagents and patient samples should be handled with care, as all materials of human origin are potentially hazardous.

The product contains the nuclide 125-iodine as an open radioactive substance. Ionising radiation in the form of photon radiation is emitted at an energy of approx. 30 keV. The half-life is 60 days. The product must be stored protected, and safeguarded against loss. It must be disposed of as radioactive waste.

To guarantee adequate radiation protection, the uptake of radioactive substances into the body should be avoided. Work rooms should be ventilated. Eating, drinking and smoking are prohibited. When working, an apron and protective gloves must be worn. Avoid remaining unnecessarily in the vicinity of radiation sources. It is recommended that the personal dose is calculated using appropriate measuring facilities such as film dosimeters.

The following kit reagents contain the preservative sodium azide at concentrations of < 0.1 percent by weight: tracer, antiserum, standards, control sera and buffer D. These reagents should not be swallowed or allowed to come in contact with the skin or mucous membranes.

Our Customer Service Department, phone: +49 (0)3302/883 300, will gladly send the reagent-specific EU Safety Data Sheets in accordance to regulation 1907/2006-EC upon request.

In the event that glass vials are included in the reagent kit, we explicitly point out that there will be a breakage hazard, and consequently a risk of injury.

The reagents must be disposed of according to the specifications of local authorities.
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Usage</th>
<th>Symbol</th>
<th>Usage</th>
<th>Symbol</th>
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<tr>
<td><img src="image" alt="Manufacturer" /></td>
<td>Manufacturer</td>
<td><img src="image" alt="CE" /></td>
<td>CE Conformity Marking According to Directive 98/79/EC on In Vitro Diagnostic Medical Devices</td>
<td><img src="image" alt="Sigma" /></td>
<td>Contains sufficient for (Number of) tests, e.g. 50</td>
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<td>Zero Standard</td>
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<td>Control Serum</td>
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<td>Consult Instruction for use</td>
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<td><img src="image" alt="Ab" /></td>
<td>Antiserum, antibody</td>
<td><img src="image" alt="BUF WASH" /></td>
<td>Washing solution</td>
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