DRG® Neopterin (EIA-1476)

USA: RUO

Revised 29 Nov. 2010 rm (Vers. 2.1)

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

PRINCIPLE OF THE TEST

The Neopterin ELISA employs an enzyme immunoassay technique to quantitate neopterin in a serum sample. Enzyme Immunoassays depend on the ability of a specific antibody to bind its corresponding antigen. To quantitate the antigen, the enzyme-conjugate (labeled) and native (unlabeled) form of the antigen compete for the limited number of binding sites on its specific antibody. As more unlabeled antigen is added to the reaction, it takes up more antibody binding sites allowing less enzyme-labeled antigen to the bound. This proceeds until equilibrium between the free and antibody-bound antigen occurs.

In the Neopterin ELISA, the sample and enzyme-conjugate are added to an antibody-coated assay well and incubated at room temperature for two hours. Following a wash step to remove all unbound labeled and unlabeled neopterin, substrate (TMB) is added and color development is allowed to proceed for thirty minutes. The enzyme reaction is then rapidly terminated with the addition of stopping solution and the absorbance is read at 450 nm. The resulting absorbance is inversely proportional to the amount of neopterin in the standard and sample.

REAGENTS PROVIDED

IMPORTANT NOTICE

Upon receipt of kit, standards and controls should be removed and stored frozen (below -15°C).

Anti-Neopterin **Coated Strip Plate**	1 x 96 Wells	
Neopterin **Standards** (8)	0.5 mL each,	0 nmol/L standard contains 1.0 mL
Neopterin **Controls** (2)	0.5 mL each	
Neopterin **Enzyme Conjugate Concentrate**	0.3 mL	
Neopterin **Enzyme Diluent**	18 mL	
Wash Buffer Concentrate	10 mL	
Color Substrate	11 mL	
Stop Solution	11 mL	

REAGENT DESCRIPTION AND PREPARATION

A. ANTI-NEOPTERIN COATED STRIP PLATES

Each plate (96 wells) is coated with a polyclonal antibody having a high affinity for neopterin.
STABILITY: Refer to the expiration date on the foil bag.
STORAGE: 2 to 8°C. Reseal foil bag after use.

Revised 29 Nov. 2010 rm (Vers. 2.1)

B. NEOPTERIN STANDARDS 1-8
Eight standards are provided at the following concentrations: 0, 0.5, 1.5, 3.0, 6.0, 12.0, 24.0, 100.0 ng/mL. These standards have been prepared in a human serum matrix containing thimerosal as a preservative.
STORAGE: Store frozen below -15°C.
STABILITY: Refer to the expiration date on the kit vial

C. NEOPTERIN CONTROL 1-2
Two controls are provided (see accompanying information for ranges). These controls have been prepared in a human serum matrix containing thimerosal as a preservative.
STORAGE: Store frozen below -15°C.
STABILITY: Refer to the expiration date on the kit vial.

D. NEOPTERIN ENZYME CONJUGATE CONCENTRATE
Neopterin-horseradish peroxidase enzyme conjugate concentrate is provided in a stabilizing buffer. The Enzyme Conjugate must be diluted 1:100 with Neopterin Enzyme Diluent prior to use (i.e. 1 part Enzyme Conjugate Concentrate + 99 parts Enzyme Diluent).
STORAGE: 2 to 8°C.
STABILITY: Concentrate: Refer to the expiration date on the kit vial.
Diluted Enzyme: Discard excess after use.

E. NEOPTERIN ENZYME DILUENT
A phosphate-buffered saline solution containing human serum, rabbit serum and thimerosal as a preservative is provided in a ready-to-use form.
STORAGE: 2 to 8°C.
STABILITY: Refer to the expiration date on the kit vial

F. WASH BUFFER CONCENTRATE
A phosphate buffered saline solution containing Tween is supplied in a 10x concentrated form.
It must be diluted 1:10 with distilled water prior to use.
STORAGE: 20°C - 25°C concentrated, 2°C - 22°C diluted.
STABILITY: Refer to the expiration date on the kit vial

G. COLOR SUBSTRATE
3,3',5,5'-Tetramethylbenzidine (TMB) is provided in a ready-to-use form.
STORAGE: 2 to 8°C.
STABILITY: Refer to the expiration date on the kit vial.

H. STOP SOLUTION
Dilute sulfuric acid solution is provided in a ready-to-use form.
STORAGE: 2 to 8°C.
STABILITY: Refer to the expiration date on the kit vial.

LIMITATIONS, PRECAUTIONS AND GENERAL COMMENTS

NOTES: (1) The serum used to manufacture the standards, controls and enzyme diluent is of human origin. Although it has been tested and found negative for HIV, HCV and HBsAg by an FDA approved method, these reagents should be handled with the same safety precautions afforded any human serum/plasma sample.
(2) These reagents may contain sodium azide, which has a tendency to build up in lead or copper plumbing, forming potentially explosive metal azides. Always flush large quantities of water through the plumbing after the disposal of these reagents.

1. Strict adherence to the protocol is advised for reliable performance. Any changes or modifications may affect the precision and accuracy of this kit and, therefore, are the responsibility of the user.
2. Neopterin is light sensitive and exposure to sunlight must be avoided. The kit reagents should be kept in the dark at 2 to 8°C when not in use.
3. Repeated freezing and thawing of specimens should be avoided. Once a sample is thawed after initial freezing, it should be assayed within twenty-four hours.
4. A standard curve must be established with every assay.
5. The reagents in this kit are designed specifically for the quantitation of neopterin in human serum. Anyone doing animal research must establish their own physiological ranges.
6. The use of grossly hemolyzed or lipemic samples should be avoided.
7. The kit reagents and materials are intended for use as an integral unit. Do not mix various lots of any component reagent within an individual run.
8. Do not use reagents after their expiration date. Once a reagent has been opened, it is considered to expire on the day the kit expires.

SPECIMEN COLLECTION AND HANDLING

Human SERUM samples may be used with the Neopterin ELISA Kit. No particular sample preparations such as fasting are required.
SPECIAL SERUM STORAGE AND HANDLING CONDITIONS ARE NECESSARY.

Serum

Draw blood into a red capped evacuated glass collection tube. Allow the blood to clot for at least thirty (30) minutes at room temperature. Separate the serum and store in the dark at 2 to 8°C if the sample is to be assayed within 24 hours. If the sample is to be assayed at a later date, store frozen below -20°C in a non-frost free freezer.
For serial determinations of different specimens from the same sample, the sample should be aliquoted after receipt. One aliquot is used for the current assay, while the second is frozen. For assaying a subsequent specimen, the frozen aliquot of the original sample is thawed and run simultaneously with the current sample. In this fashion, valid comparisons can be made regarding shifts or trends in the neopterin concentrations observed between specimens.

EQUIPMENT AND REAGENTS REQUIRED

In addition to the materials provided with this kit, the following supplies are required:

- A pipet that can accurately and precisely deliver the required volumes (25, 100, 200 or 300 µL).
- A rotary horizontal shaker.
- A microplate reader capable of reading absorbance at 450 nm.

Revised 29 Nov. 2010 rm (Vers. 2.1)

ASSAY PROCEDURE

ASSAY PREPARATION

1. Bring all standards, controls, samples, plates and reagents to room temperature prior to use.
2. Place the required number of Anti-Neopterin coated strips in the frame provided. Reseal the unused strips in the foil bag along with the desiccant and refrigerate.

ASSAY STEPS (Duplicates recommended)

1. Add 25 µL each of NEOPTERIN STANDARDS, CONTROLS and SAMPLES to the appropriate wells of the microtiter strip plate. Prepare NSB wells with 25 µL of "0" ng/mL standard and 100 µL of NEOPTERIN ENZYME DILUENT.
2. Add 100 µL of diluted ENZYME CONJUGATE to all wells except NSB.
3. Cover plate and incubate at room temperature for TWO (2) HOURS on a rotary horizontal shaker (200 rpm is recommended).
4. Aspirate contents of all wells or decant and blot plate on absorbent paper.
5. Wash wells three times with 200-300 µL of diluted WASH BUFFER and aspirate or decant to dryness.
6. Add 100 µL of COLOR SUBSTRATE to all wells and incubate for THIRTY (30) MINUTES on a rotary horizontal shaker.
7. Add 100 µL STOPPING SOLUTION to all wells.
8. Read absorbance at 450 nm and calculate the results using the supplied formula or a data reduction system.

QUALITY CONTROL

The controls provided with this kit or serum pools containing different concentrations of neopterin should be assayed routinely as unknowns. The concentrations of these controls should be plotted on a run to run basis using a Levey-Jennings type system in order to assess the performance and reliability of the assay. For further information see:
DAVID RODBARD: "Statistical Quality Control and Routine Data Processing for Radioimmunoassays and Immunoradiometric Assays." CLIN CHEM 20/10, 1255-1270 (1974).

PROTOCOL

Wells	Sample Description	Sample Volume	Enzyme Conjugate	Incubate	Wash Buffer	Color Substrate	Incubate	Stop Solution	Read
A1, B1	NSB	25 µL "0"	(100 µL)*	Shake Two Hours at Room Temp.	Wash Three Times With 200 µL 300 µL ↓	100 µL	Shake 30 Min. at Room Temp.	100 µL	Read at 450 nm ↓
C1, D1	0 ng/mL	25 µL	100 µL			↓		↓	
E1, F1	0.5 ng/mL	↓	↓						
G1, H1	1.5 ng/mL								
A2, B2	3.0 ng/mL								
C2, D2	6.0 ng/mL								
E2, F2	12.0 ng/mL								
G2, H2	24.0 ng/mL								
A3, B3	100 ng/mL								
C3. D3	Control I								
E3, F3	Control II								
G3, H3	Sample								

* 100 µL enzyme diluent

CALCULATIONS

1. Average the absorbance of all duplicates. Subtract the averaged non-specific binding (NSB) absorbance from the averages obtained above.
   This yields the net absorbance. Divide the net absorbance by the net zero standard absorbance (Bo) to obtain the percent bound (% B/Bo).
2. FORMULA:

$$B/Bo = \frac{\text{Abs. (sample)} - \text{Abs. (NSB)}}{\text{bs. (zero standard)} - \text{Abs. (NSB)}} \times 100$$

Abs. = average absorbance of duplicate wells
NSB = non-specific binding (also known as the blank)
Sample = particular serum or standard being calculated
Zero Standard = 0 ng/mL standard or 100% binding wells

3. Construct a plot of the percent bound (Y-axis) versus the concentration of the neopterin standards ((X-axis) starting with the 0.5 ng/mL point. Either logit-log or semi-log graph paper may be used. This yields the standard curve.
4. Using the standard curve, determine the neopterin concentration of each sample.
   NOTE: Values that bind either higher or lower than the standard curve should not be determined by extrapolation.

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COMPUTER ASSISTED DATA REDUCTION

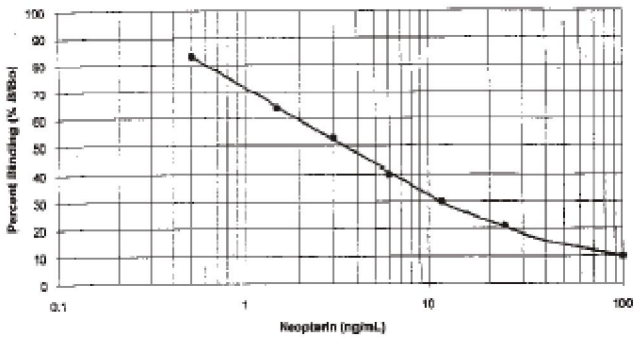
Automated data reduction may be used with a variety of curve fitting algorithms. A smooth curve fit on semi-log or a linear curve fit on logit-log graph paper is recommended. To program your automated data reduction system, please contact your software manufacturer.

SAMPLE ASSAY

These calculations are for example only. The user must construct a standard curve each time the assay is run.

SAMPLE	AVERAGE ABSORBANCE	NET ABSORBANCE	%B/Bo	NEOPTERIN (ng/mL)
NSB	0.104			
0 ng/mL	2.301			
0.5 ng/mL	1.929	1.825	83	
1.5 ng/mL	1-509	1.405	64	
3 ng/mL	1.277	1.173	53	
6 ng/mL	0.993	0.889	40	
12 ng/mL	0.769	0.665	30	
24 ng/mL	0.569	0.465	21	
100 ng/mL	0.333	0.229	10	
Control 1	1.615	1.511	69	1.2
Control 2	1.054	0.950	43	5.1

SAMPLE STANDARD CURVE NOTE: This standard curve serves only as an example.
Sample concentrations must not be derived from it.



Revised 29 Nov. 2010 rm (Vers. 2.1)

INTRA-ASSAY VARIATION

Sample	N	Neopterin Concentration (nq/mL)	S.D.	C.V. (%)
1	12	2.1	0.2	9.5
2	12	6.4	0.5	7.8
3	12	7.1	0.4	5.6
4	12	13.6	0.8	5.9
5	12	23.1	1.2	5.2

INTER-ASSAY VARIATION

Sample	N	Neopterin Concentration (nq/mL)	S.D.	C.V. (%)
1	12	1.8	0.1	5.6
2	12	2.8	0.1	3.6
3	12	5.4	0.3	5.6
4	12	13.1	0.9	6.9
5	12	25.8	2.1	8.1

SPECIFICITY OF THE ANTISERIUM

The following ligands have been checked for cross reactivity with this antiserum. The percentages indicate the cross reactivity at 50% displacement as compared to the neopterin curve.

COMPOUND	% CROSS REACTION
Neopterin	100
7,8-Dihydro-D-Neopterin	3.90
Monapterin	1.30
Biopterin	0.03
lsoxanthopterin	0.02
7,8-Dihydro-D-Biopterin	< 0.01
5,6,7,8-Tetrahydrobiopterin	< 0.01
Xanthopterin	< 0.01

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