SKU IP204116

ELISA KIT FOR HUMAN NEOPTERIN

Research Use Only
For *in vitro* applications - not for consumption

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VERSION 1.0

INTENDED USE

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of Neopterin in human serum, cell culture supernates, and other biological fluids.

KIT CONTENTS & STORAGE

Microelisa Plate [12 X 8 Strips]
Human Neopterin Standard [0.5mL]
HRP-Conjugated Human Neopterin detection antibody [6mL]
Standard Diluent [1.5mL]
Sample Diluent [6ml]
Chromogen Solution A and B [6mL each]
30X Wash solution [20mL]
Stop Solution [6mL]
Plate Sealing Membrane [2]
User manual [1pc]

The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20°C for unused kit while the others should be at 4°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

ELISA Reader, 37 °C Thermostat, micropipettes and tips, Eppendorf tubes, absorbent paper, autowasher, deionized or distilled water.

SUMMARY

This kit is based on the principle of antibody sandwich enzyme linked immunosorbent assay. In brief, samples (and standards) containing the analyte are added to the micro-titre wells that are pre-coated with a protein which captures the analyte. Subsequently HRP-conjugated reagent(s) is(are) added to form an immune complex. This is followed by incubation and washing, removal of unbound enzyme, and sequential addition of chromogen which turns blue and finally changes into yellow color under the effect of acid. The color density is positively correlated with the concentration of analyte.

CHARACTERISTICS

Sample Type: Serum, plasma, tissue homogenates, cell culture supernatants and other biological fluids.

Detection Range: 45 pg/mL - 720 pg/mL
Minimum Detection Dose : 4.0pg/mL
Intra-Assay Precision: CV<10%
Inter Assay Precision: CV<12%
Linearity with spiked analytes: ρ=0.99
Cross Reactivity: None observed with analogues
Validity: 1 year from the date on manufacture
Storage: 2-8°C

Once Opened unused wells should be put into the sealed bag with a desiccant pack and may be stored for up to 1 month at 2-8 °C.

SAMPLE COLLECTION

Tissue homogenates - Rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces and homogenized in PBS with a glass homogenizer on ice. Different lysis buffer needs to be chosen based on subcellular location of the target protein. 1mL lysis buffer should be added in 9 mg tissue sample. Protease inhibitor is recommended to be added into the PBS. To further break the cells, sonication of the suspension may be done with an ultrasonic cell disrupter or they may be subjected to freeze-thaw cycles. The homogenates are centrifugated for 5 minutes at 10,000×g to collect supernatant.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Collect the supernates and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Cell Lysates - Cells need to be lysed before assaying according to the following directions. 1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000×g for 5 minutes (suspension cells can be collected by centrifugation directly). 2. Wash cells three times in cold PBS. 3. Resuspend cells in fresh lysis buffer with concentration of 107 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified. 4. Centrifuge at 1,500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤-20°C.

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

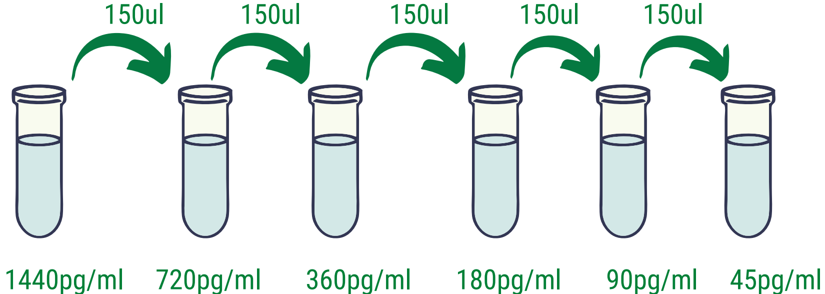
Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Note: Samples should be centrifuged adequately and no hemolysis or granule is to be allowed.

REAGENT PREPARATION

1. Bring all kit components and samples to room temperature before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.
Wash Buffer- Dilute with Distilled or deionized water 1:20 (48T) /1:30 (96T).

2. **Standard** - Pipette 150 µL of Standard Diluent into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the highest standard. Standard Diluent serves as the zero standard.



Note: If samples generate values higher than the highest standard, please dilute the samples with Sample Diluent and repeat the assay.

PROCEDURE

1.Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the microplate.

2.Add standard: Set Standard wells, testing sample wells. Add diluted standard 50 μL to standard well.

3.Add Sample: Add sample diluent 40 μL to testing sample well. Then add sample 10 μL to testing sample well. In blank well don't add anything.

4.Cover with a plate cover and incubate for 45 minutes at 37 °C.

5.Aspirate each well and wash, repeating the process four times for a total of five washes, 1-3 minutes per time. Wash by filling each well with Wash buffer (250 μL)using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid ateach step is essential to good performance. After the last wash, remove any remaining Wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6.Add HRP-Conjugated detection antibody 50 μL to each well, except blank well.

7.Cover with plate cover. Incubate for 30 minutes at 37 °C.

8.Repeat the aspiration/wash process for five times as in step 5.

9.Add chromogen solution A 50 μL and chromogen solution B 50 μL to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light.

10.Add 50 μL Stop Solution to each well. The color in the wells should change from blueto yellow. If the color in the wells is green or the color change does not appear uniform,gently tap the plate to ensure thorough mixing.

11.Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15minutes.

CALCULATION

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4- PL) curve fit.

As an alternative, construct a standard curve by plotting the concentration on the x-axis against the mean absorbance for each standard on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the analyte concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TROUBLESHOOTING

We understand research is challenging as it is but it can get very frustrating when experiments themselves don't go as they should. That is the precise reason why we strive to ensure that all our products work for the application shown in their respective data sheets.

We have compiled a list of most common errors and resolutions on our technical support webpage www.immunoport.com/scientific-support. Still if you have any concerns please feel free to write to us at info@immunoport.com

PRECAUTIONS

- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.
- Do not mix or substitute reagents with those from other lots or sources.
- Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37°C directly.
- Chromogen Solution is easily contaminated. If bluish prior to use, do not use.
- Stop Solution should be added to the plate in the same order as the Chromogen solution. The color developed in the wells will turn from blue to yellow upon addition ofthe Stop Solution. Wells that are green in color indicate that the Stop Solution has notmixed thoroughly with the Chromogen solution.
- Serum and plasma should be handled as potentially hazardous and capable oftransmitting disease. Disposable gloves must be worn during the assay procedure,since no known test method can offer complete assurance that products derived fromRat blood will not transmit infectious agents. Therefore, all blood derivatives should beconsidered potentially infectious and good laboratory practices should be followed.
- If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals are completely dissolved.
- Add sodium hypochlorite to a final concentration of 1.0% in the liquid waste generated. The waste should be allowed to stand for a minimum of 30 minutes to inactivate any viruses before disposal.

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