BioAim Scientific Inc

Human IL-6 EasyTest™ ELISA Kit

Cat.No: 1010018

Instruction Manual

For research use only

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I. INTRODUCTION

Interleukin 6 (IL-6) is a pleiotropic cytokine produced by a variety of cells. It acts on a wide range of tissues, exerting growth-induction, growth-inhibition, and differentiation respectively, depending on the nature of the target cells. IL-6 is involved in B-cell differentiation, acute phase protein induction in liver cells, growth promotion of myeloma cells, induction of IL-2 and IL-2 receptor expression, proliferation and differentiation of T cells, enhancement of IL-3-induced multipotential colony cell formation in hematopoietic stem cells.

Measurement of IL-6 levels in serum and other body fluids can provide more detailed insights into the pathological situations in bacterial and viral infections, autoimmune diseases, proliferative diseases and some solid tumors.

The BioAim Human IL-6 EasyTest™ ELISA kit can quantitatively measure IL-6 in human serum or plasma. It is a simple and rapid technology for the quantitation of antigen in a range of sample matrices. The whole process takes less than 1.5 hours with high accuracy and precision. EasyTest™ ELISA is faster and easier to perform than standard format ELISA with less reagent handling and fewer pipetting steps.

II. REAGENTS

1. Human IL-6 Microplate: 96 breakable wells (12strips x 8wells) coated with anti-human IL-6.
2. 20x Wash Buffer Concentrate: 1 Vial, 25 ml.
3. 5x Assay Diluent: 1vial, 15 ml.
4. Standards: 10µl/ vial, 2 vials, recombinant human IL-6.
5. BioAim human IL-6 Mix: 9µl/vial, 4 vials.
6. TBM Substrate solution: 1 Vial, 12 ml.
7. Stop Solution: 1 Vial, 8 ml of 0.2 M sulfuric acid.

III. STORAGE

1. The kit can be stored for up to 6 months at 2º to 8ºC from the date of shipment.
2. Standard can be stored at -20 ºC or -80 ºC. Use freshly prepared standard within 12 hours (stored at 2~8ºC).
3. Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8ºC. Return unused strip to the pouch containing desiccant pack, reseal along entire edge and keep in 2~8ºC.
4. Avoid repeated freeze-thaw cycles.

IV. ADDITIONAL MATERIALS REQUIRED

1. Distilled or deionized water.
2. Precision pipettes, with disposable plastic tips.
3. Beakers, flasks, cylinders necessary for preparation of reagents.
4. Microplate washing device (multichannel pipette or automated microplate washer).
5. Microplate shaker.
6. Microplate reader capable of reading at 450 nm.

V. PRECAUTIONS

1. All reagents must be at room temperature (18ºC to 25ºC) before running assay.
2. Do not mix or substitute reagents with those from other lots or other sources.
3. Do not use kit reagents beyond expiration date on label.
4. Do not expose kit reagents to strong light during storage or incubation.
5. Use disposable pipette tips for each transfer to avoid microbial contamination or cross contamination of reagents.
6. Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results.
7. Avoid contact of stop solution with skin or eyes. If contact occurs, immediately flush area with copious amounts of water.
8. Do not use TMB substrate solution if it has begun to turn blue.
9. Do not expose bleach to work area during actual test procedure because of potential interference with enzyme activity.

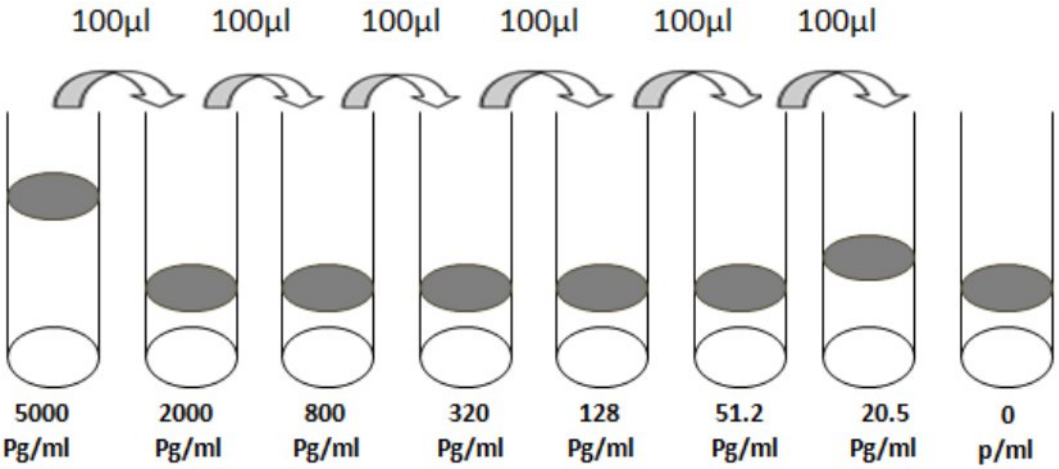
VI. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18~25°C) before use.
2. **Assay diluent**
   Dilute the concentrated assay diluent 1:5 with distilled water (e.g. 10ml plus 40ml).
3. **Wash Buffer**
   Dilute the concentrated wash buffer 1:20 with distilled water (e.g. 20ml plus 380ml).
4. **Sample**
   Levels of the target protein may vary among different specimens. Optimal dilution factors for each sample must be determined by the investigator.

The dilution scheme is only suggestion: the recommended dilution for serum and plasma is 1: 2.

5. Standard

a. Briefly spin standard vial before use. Add 190 µl 1x Assay Diluent to prepare a 50ng/ml standard. Gently vortex to mix.
b. Take 50 µl IL-6 standard into a tube; then add 450 µl 1x Assay Diluent to prepare a 5000 pg/ml stock standard solution.
c. Add 150 µl 1x Assay Diluent to 7 tubes. Label as 2000pg/ml, 800pg/ml, 320pg/ml, 128pg/ml, 51.2pg/ml, 20.5pg/ml and the last tube with 1x assay diluent is the blank as 0pg/ml.
d. Perform serial dilutions by adding 100 µl of each standard to the next tube and vortexing between each transfer (see figure below).



6. BioAim human IL-6 Mix

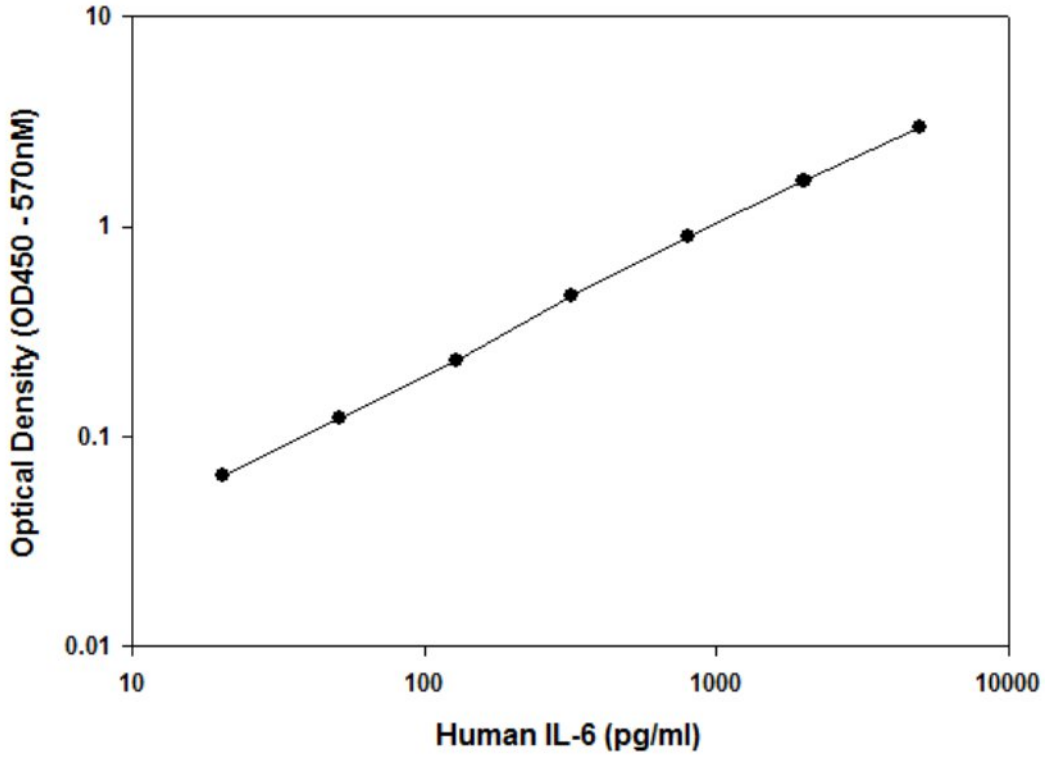
Within15minutes prior to use, briefly spin the vial. Add 1490 µl of 1x Assay diluent to the vial and mix by pipetting. A vial mix can be used for around 30 wells.

VII. ASSAY PROCEDURE

1. All reagents must be brought to room temperature (18-25°C) prior to use. Place the required number of microwells in the holder. It is recommended that all samples, standards, and blanks be run in duplicate.
2. Add 50 µl of 1x Assay Diluent into the blank wells.
3. Add 50 µl of each standard (*see reagent preparation step 5*) and samples into the designated wells. Gently shake/tap the plate for 5 seconds to mix.
4. Add 50 µl of BioAim IL-6 Mix into all wells, including the blank wells.
5. Cover wells with plate sealer and incubate at room temperature (18~25°C) for 1 hour with gentle shaking.
6. Decant or aspirate contents of wells. Wash wells by filling with at least 300 µl/well prepared wash buffer followed by decanting/aspirating. Soak wells in wash buffer for 30 seconds to 1 minute for each wash. Repeat wash 4 times for a total of 5 washes. After the last wash, blot plate on absorbent paper to remove residual buffer. Thorough washing at this step is very important, complete removal of liquid is required for proper performance.
7. Pipette 100 µl of TMB Substrate Solution to each well. Incubate plate for 15 minutes at room temperature in the dark with gentle shaking.
8. Add 50 µl of stop solution to each well.
9. Read absorbance at 450nm within 30 minutes of stopping reaction. If wavelength correction is available, subtract the optical density readings at 570nm from readings at 450nm.

VIII. CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of duplicate standards, samples and controls. Subtract the average zero standard optical density.
2. Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the IL-6 concentration on log-log graph paper or using Sigma plot software. Draw a best fit curve through the points of the graph.
3. To determine the concentration of circulating IL-6 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding IL-6 concentration.
4. A representative standard curve is shown below. This standard curve is for demonstration only. A standard curve must be run with each assay by operator.



IX. PERFORMANCE

A. Sensitivity

The minimum detectable dose of IL-6 was determined to be less than 1pg/ml. This is defined as two standard deviations above the mean optical density of 20 replicates of the zero standards.

B. Recovery

Recovery was determined by spiking various levels of Human IL-6 into the diluted sample types listed below. Mean recoveries are as follows:

Sample Type	Average % recovery	Range %
Serum	118	108-126
Plasma	104	97-108

C. Linearity

Sample	Dilution	% of expected
Seum	1:2	106
	1:4	109
	1:8	103
Plasma	1:2	111
	1:4	110
	1:8	109

D. Specificity

No cross-reactivity was identified with the following cytokines: Adiponectin, Angiopoietin-1, BDNF, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IP-10, G-CSF, GM-CSF, IFN-gamma, Leptin, MCP-1, PDGF, RANTES, SCF, TGF-beta, TIMP-2, TNF-alpha, TNF-beta, and VEGF.

E. Reproducibility
Intra-Assay CV%: <10%
Inter-Assay CV%: <15%

X. REFERENCES

1. Hack C. E., etal. (1989). Blood 74, 1704.
2. Nijsten M. W. N., etal (1987). Lancet II, 921ff.
3. Oka Y., A. etal. (1992). Cytokine 4, 298–304.

XI. Troubleshooting

Problem	Cause	Solution
1.Poor standard curve	1. Inaccurate pipetting 2. Improper standard dilution	1. check pipettes; 2. Ensure briefly spin the vial of standard, take the right amount to dilution.
2. Low signal	1. Too brief incubation time 2. Inadequate reagent volumes or improper dilution	1. ensure adequate incubation time; 2. Check pipettes and ensure corrected preparation.
3. Large CV	Inaccurate pipetting	1. Check pipettes; 2. Accurately perform each step.
4.High background	1. Plate is insufficiently washed; 2. Wash buffer contamination	1.Follow the manual correctly; if using a plate washer, check that all ports are working functionally; 2. Prepare fresh buffer.
5. Low sensitivity	1.ELISA kit improper storage 2. Stop solution	1. Follow the manual to store each component correctly; 2. Add enough stop solution to each well.

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