Datasheet for ABIN625026

# IL-6 ELISA Kit

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## Overview

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| Quantity: | 96 tests |
| Target: | IL-6 (IL6) |
| Reactivity: | Human |
| Method Type: | Sandwich ELISA |
| Detection Range: | 3-1000 pg/mL |
| Minimum Detection Limit: | 3 pg/mL |
| Application: | ELISA |

## Product Details

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| Purpose: | Human IL-6 ELISA Kit for cell culture supernatants, plasma, and serum samples. |
| Sample Type: | Plasma, Cell Culture Supernatant, Serum |
| Analytical Method: | Quantitative |
| Detection Method: | Colorimetric |
| Specificity: | This ELISA kit shows no cross-reactivity with any of the cytokines tested: Human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, G-CSF, GM-CSF, IFN-gamma, Leptin (OB), MCP-1, MCP-2, MCP-3, MDC, MIP-1 alpha, MIP-1 beta, MIP-1 delta, PARC, PDGF, RANTES, SCF, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF. |
| Sensitivity: | < 3 pg/mL |
| Characteristics: | • Strip plates and additional reagents allow for use in multiple experiments |

## Product Details

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| | • Quantitative protein detection<br>• Establishes normal range<br>• The best products for confirmation of antibody array data |
| Components: | • Pre-Coated 96-well Strip Microplate<br>• Wash Buffer<br>• Stop Solution<br>• Assay Diluent(s)<br>• Lyophilized Standard<br>• Biotinylated Detection Antibody<br>• Streptavidin-Conjugated HRP<br>• TMB One-Step Substrate |
| Material not included: | • Distilled or deionized water<br>• Precision pipettes to deliver 2 µL to 1 µL volumes<br>• Adjustable 1-25 µL pipettes for reagent preparation<br>• 100 µL and 1 liter graduated cylinders<br>• Tubes to prepare standard and sample dilutions<br>• Absorbent paper<br>• Microplate reader capable of measuring absorbance at 450nm<br>• Log-log graph paper or computer and software for ELISA data analysis |

## Target Details

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| Target: | IL-6 (IL6) |
| Alternative Name: | IL-6 (IL6 Products) |
| Background: | IL-6 is a multi-functional cytokine that induces a number of biological responses and plays a role in cell growth regulation, immune host defense, angiogenesis and others. It is produced by many different cell types including monocytes, fibroblasts, endothelial cells, glial cells, and keratinocytes. Human IL-6 is a 20.5 kD protein containing 184 amino acid residues. The Human IL-6 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human IL-6 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human IL-6 coated on a 96-well plate. Standards and samples are pipetted into the wells and IL-6 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human IL-6 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IL-6 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the |

## Target Details

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| | color is measured at 450 nm. Reproducibility: Intra-Assay: CV<10% Inter-Assay: CV<12. |
| Gene ID: | 3569 |
| UniProt: | P05231 |
| Pathways: | TLR Signaling, Hormone Transport, Negative Regulation of Hormone Secretion, Myometrial Relaxation and Contraction, Positive Regulation of Immune Effector Process, Production of Molecular Mediator of Immune Response, Regulation of Carbohydrate Metabolic Process, Autophagy, Cell RedoxHomeostasis, Cancer Immune Checkpoints, Inflammasome |

## Application Details

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| Application Notes: | Recommended Dilution for serum and plasma samples2 fold |
| Sample Volume: | 100 µL |
| Plate: | Pre-coated |
| Protocol: | 1. Prepare all reagents, samples and standards as instructed in the manual.<br>2. Add 100 µL of standard or sample to each well.<br>3. Incubate 2.5 h at RT or O/N at 4 °C.<br>4. Add 100 µL of prepared biotin antibody to each well.<br>5. Incubate 1 h at RT.<br>6. Add 100 µL of prepared Streptavidin solution to each well.<br>7. Incubate 45 min at RT.<br>8. Add 100 µL of TMB One-Step Substrate Reagent to each well.<br>9. Incubate 30 min at RT.<br>10. Add 50 µL of Stop Solution to each well.<br>11. Read at 450 nm immediately. |
| Reagent Preparation: | 1. Bring all reagents and samples to room temperature (18-25 °C) before use.<br>2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of culture supernatants and urine. Suggested dilution for normal serum/plasma: 2 fold*. *Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.<br>3. Assay Diluent B should be diluted 5-fold with deionized or distilled water.<br>4. Briefly spin the vial of Item C and then add 500 µL Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium and urine) into Item C vial to prepare a 12,000 pg/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 40 µL IL-6 standard from the vial of Item C, into a tube with 440 µL Assay Diluent A or 1x Assay Diluent B |

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| | to prepare a 1000 pg/mL standard solution. Pipette 400 µL Assay Diluent A or 1x Assay Diluent B into each tube. Use the 1000 pg/mL standard solution to produce a dilution series . Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/mL). 4 00 µL 40 µL standard + 440 µL 200myl 200 µL 200 µL 200 µL 200 µL 1000 333.3 111.1 37.04 12.35 4.12 1.37 0 pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL<br>5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.<br>6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure.<br>7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 600-fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 25 µL of HRP-Streptavidin concentrate into a tube with 15 ml 1x Assay Diluent B to prepare a final 600 fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well. 5 RayBio® Human IL-6 ELISA Kit Protocol |
| Assay Procedure: | 1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. It is recommended that all standards and samples be run at least in duplicate.<br>2. Add 100 µL of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking.<br>3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 myl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each 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.<br>4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.<br>5. Discard the solution. Repeat the wash as in step<br>6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.<br>7. Discard the solution. Repeat the wash as in step<br>8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 |

## Application Details

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| | minutes at room temperature in the dark with gentle shaking. 6 . Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately. |
| Calculation of Results: | Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points. 7 RayBio® Human IL-6 ELISA Kit Protocol<br>Typical Data: These standard curves are for demonstration only. A standard curve must be run with each assay. Assay Diluent A Human IL-6 concentration (pg/mL) 0.1 1 10 100 1000 10000 O D =4 50 n m 0.01 0.1 1 10 Assay Diluent B Human IL-6 concentration (pg/mL) 0.1 1 10 100 1000 10000 O D =4 50 n m 0.01 0.1 1 10<br>Sensitivity: The minimum detectable dose of IL-6 is typically less than 3 pg/mL.<br>Recovery: Recovery was determined by spiking various levels of human IL-6 into human serum, plasma and cell culture media. Mean recoveries are as follows: Sample Type Average % Recovery Range ( %) Serum 92.63 83-103 Plasma 93.74 84-104 Cell culture media 95.65 83-105 8 RayBio® Human IL-6 ELISA Kit Protocol<br>Linearity: Sample Type Serum Plasma Cell culture media 1:2 Average % of Expected 95 96 97 Range ( %) 84-103 85-105 84-105 1:4 Average % of Expected 96 95 97 Range ( %) 83-105 84-103 85-105<br>Reproducibility: Intra-Assay: CV<10 % Inter-Assay: CV<12 % |
| Assay Precision: | Intra-Assay: CV< 10 % Inter-Assay: CV< 12 % |
| Restrictions: | For Research Use only |

## Handling

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| Handling Advice: | Avoid repeated freeze-thaw cycles. |
| Storage: | -20 °C |
| Storage Comment: | The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. |
| Expiry Date: | 6 months |

## Publications

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| Product cited in: | Avenoso, DAscola, Scuruchi, Mandraffino, Campo, Campo: "miR146a up-regulation is involved |

in small HA oligosaccharides-induced pro-inflammatory response in human chondrocytes." in: **Biochimica et biophysica acta. General subjects**, pp. 129731, (2020) (PubMed).

Bassyouni, El-Wakd, Azab, Bassyouni: "Diminished soluble levels of growth arrest specific protein 6 and tyrosine kinase receptor Axl in patients with rheumatoid arthritis." in: **International journal of rheumatic diseases**, Vol. 20, Issue 1, pp. 53-59, (2018) (PubMed).

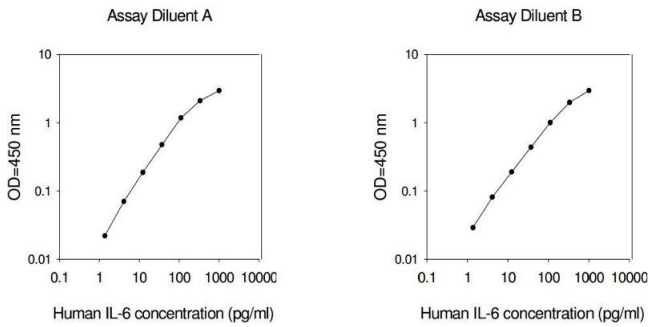
Feng, Su, Luo, Jing, Yi: "Serum levels of C1q/tumor necrosis factor-related protein-1 in children with Kawasaki disease." in: **Pediatric research**, Vol. 83, Issue 5, pp. 999-1003, (2018) (PubMed).

Liu, Sun, Tao, Xu, Xu, Cheng, Wang, Zhang: "Pyrroloquinoline Quinone Decelerates Rheumatoid Arthritis Progression by Inhibiting Inflammatory Responses and Joint Destruction via Modulating NF-κB and MAPK Pathways." in: **Inflammation**, Vol. 39, Issue 1, pp. 248-56, (2016) ( PubMed).

Shih, Janckila, Lee, Chou, Huang, Kwok, Ho, Chao: "Effects of bariatric weight loss surgery on glucose metabolism, inflammatory cytokines, and serum tartrate-resistant acid phosphatase 5a in obese Chinese adults." in: **Clinica chimica acta; international journal of clinical chemistry**, Vol. 453, pp. 197-202, (2016) (PubMed).

There are more publications referencing this product on: Product page

## Images




**ELISA**

**Image 1.**