Human IL-6 (Interleukin 6) ELISA Kit

Cat. No. KITG-094 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The Human IL-6 (Interleukin 6) ELISA Kit is designed for the quantitative determination of human interleukin 6 (IL-6) in serum, plasma, cell culture supernatant, tissue samples, and other compatible biological samples. The assay uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) format. The kit is available in 48T and 96T formats. The detection range is 1.5-48 pg/mL, and the minimum detectable dose is less than 0.1 pg/mL. Intra-assay precision is CV < 10%, inter-assay precision is CV < 15%, and recovery is 85%-115%.
Species	Human
Description	Assay Principle The microplate is pre-coated with an anti-human IL-6 capture antibody. Human IL-6 calibrators and test samples are added to the wells, followed by an HRP-labeled anti-human IL-6 detection antibody. After incubation and thorough washing to remove unbound components, a capture antibody-antigen-HRP-labeled detection antibody sandwich complex is formed on the solid surface of the microplate. Substrates A and B are added. Under HRP catalysis, a blue product develops and is converted to yellow after addition of the acidic stop solution. Absorbance is measured at 450 nm. The optical density is positively correlated with the human IL-6 concentration in the sample. The sample concentration is calculated from the fitted calibrator curve. Performance Characteristics Detection range: 1.5-48 pg/mL. Sensitivity: Minimum detectable dose < 0.1 pg/mL. Precision: Intra-assay CV < 10%; inter-assay CV < 15%. Recovery: 85%-115%. Specificity: The kit recognizes natural and recombinant human IL-6 and shows no cross-reactivity with structurally similar analogues. Format: 48T or 96T.

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

Form	Kit
Applications	Quantitative measurement of human IL-6 in serum, plasma, cell culture supernatant, cell lysate, tissue homogenate, urine, and other compatible biological samples.
Notes	Kit Components and Storage After Opening Pre-coated microplate: 48T for the 48T kit or 96T for the 96T kit; store at 2-8°C for up to 14 days after opening. Calibrators: 0.3 mL × 6 tubes for either kit size; store at 2-8°C for up to 14 days after opening. Calibrator concentrations are 48, 24, 12, 6, 3, and 1.5 pg/mL. Sample Diluent: 3 mL for 48T or 6 mL for 96T; store at 2-8°C for up to 180 days after opening. HRP-Labeled Detection Antibody: 5 mL for 48T or 10 mL for 96T; store at 2-8°C for up to 14 days after opening. Color-Developing Substrate A: 3 mL for 48T or 6 mL for 96T; store at 2-8°C for up to 180 days after opening. Color-Developing Substrate B: 3 mL for 48T or 6 mL for 96T; store at 2-8°C for up to 180 days after opening. Stop Solution: 3 mL for 48T or 6 mL for 96T; store at 2-8°C for up to 180 days after opening. 20× Wash Solution: 15 mL for 48T or 25 mL for 96T; store at 2-8°C for up to 180 days after opening. Plate Sealing Film: 2 sheets. Instruction Manual: 1 copy. Resealable Bag: 1. Before use, verify that the reagent labels and quantities match the kit configuration. If components will be reused, ensure they were not contaminated during the previous use. Unused microplate strips must be resealed and stored at 2-8°C. Materials Required but Not Supplied Microplate reader capable of measuring absorbance at 450 nm; pipettes and pipette tips; reagent reservoir; 37°C incubator or water bath; test tubes, centrifuge tubes, graduated cylinders, and other vessels for reagent preparation; distilled or deionized water; vortex mixer; microplate shaker. Important Precautions 1. Use the kit only within the stated expiration period. Do not use expired reagents. 2. Do not mix this kit or its components with kits or components from other manufacturers. Use the sample diluent supplied with the kit. 3. If a sample value is above the highest calibrator concentration, dilute the sample appropriately and repeat the assay. 4. Human anti-mouse antibodies or other heterophilic antibodies in samples may interfere with the assay. Evaluate and eliminate this source of

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

interference before testing. 5. Results obtained with other methods are not directly comparable with results obtained using this kit. 6. Wear a laboratory coat and latex gloves. When testing blood or other body-fluid samples, follow applicable biosafety requirements. 7. Strictly follow the specified incubation times and temperatures. Bring all reagents to room temperature, 20-25°C, before use and return them to refrigerated storage immediately after use. 8. Improper washing may produce inaccurate results. Before adding substrate, remove as much residual liquid as possible. Do not allow the microwells to dry during incubation or between washing and the next step. 9. Remove residual liquid and fingerprints from the bottom of the plate because they may affect optical-density readings. 10. The substrate color solution should be colorless or only very lightly colored before use. 11. Avoid cross-contamination among reagents and specimens. 12. Protect reagents and the plate from direct strong light during storage and incubation. 13. The microplate reader must have a 450 ± 10 nm filter and an optical-density range of 0-3.5. Preheat the reader for 15 minutes before use. 14. Use disposable microcentrifuge tubes and pipette tips, and do not interchange them among reagents or samples. Sample Preparation and Storage The following procedures are general guidelines. Do not use sodium azide as a preservative during sample collection or storage. If samples are not analyzed immediately, aliquot and freeze them, and avoid repeated freeze-thaw cycles. Cell Culture Supernatant: Centrifuge to remove precipitates. Analyze immediately or aliquot and store at -20°C. Serum: Collect blood in a clean tube, allow it to clot for 30 minutes at room temperature, centrifuge at 2000 × g for 20 minutes, and collect the serum. Analyze immediately or aliquot and store at -20°C. Plasma: Collect blood using heparin, citrate, or EDTA as anticoagulant. Within 30 minutes after collection, centrifuge at 2000 × g for 20 minutes at 2-8°C. To minimize platelet effects, an additional centrifugation at 10,000 × g for 10 minutes at 2-8°C is recommended. Analyze immediately or aliquot and store at -20°C. Cell Lysate: For adherent cells, remove the culture medium and wash once with PBS, physiological saline, or serum-free medium. Add an appropriate volume of lysis buffer and pipette several times to ensure full contact between the lysis buffer and cells. Cells are generally lysed after

approximately 10 seconds. For suspension cells, collect the cells by centrifugation, wash once with PBS, physiological saline, or serum-free medium, add an appropriate volume of lysis buffer, disperse the cells by pipetting, and gently flick the tube to complete lysis. After complete lysis, centrifuge at 10,000-14,000 × g for 3-5 minutes and collect the supernatant. Analyze immediately or aliquot and store at -20°C. Tissue Homogenate: Rinse the tissue with pre-cooled PBS, 0.01 M, pH 7.4, to remove residual blood because lysed red blood cells may affect the result. Weigh and mince the tissue. Add PBS at an approximate tissue-weight-to-buffer-volume ratio of 1:9, for example 1 g tissue to 9 mL PBS. The volume may be adjusted according to experimental requirements and must be recorded. Addition of a protease inhibitor to PBS is recommended. Homogenize thoroughly on ice in a glass homogenizer. Further cell disruption may be achieved by sonication or repeated freeze-thaw cycles. Centrifuge the homogenate at 5000 × g for 5-10 minutes and collect the supernatant for testing. Urine: Collect in a sterile tube and centrifuge at 2000 × g for 20 minutes. Carefully collect the supernatant. If precipitate forms, centrifuge again. Troubleshooting Poor Standard-Curve Gradient: Inaccurate aspiration or dispensing - check the pipette and tips. Insufficient equilibration time - allow sufficient equilibration. Incomplete washing - ensure the required washing time, number of cycles, and fill volume per well. Weak or No Color Development: Insufficient incubation - use the specified incubation time. Incorrect assay temperature - use the recommended temperature. Insufficient or omitted reagent volume or incorrect dilution - check all aspiration and dispensing steps and add every reagent in the correct sequence and amount. Inactive enzyme conjugate or failed substrate - mix the enzyme conjugate with substrate and confirm rapid color development. Low Readings: Incorrect microplate-reader settings - verify wavelength and filter settings and preheat the reader. High Coefficient of Variation: Incorrect dispensing - check the pipetting process. High Background: Detection-antibody working concentration too high - use the recommended dilution. Incomplete washing - ensure complete washing at every step; when using an automatic washer, check all outlets for blockage and confirm use of the kit wash solution. Contaminated wash solution - prepare fresh wash solution.

	Low Sensitivity: Improper kit storage - store all reagents as instructed. Reading before the reaction has been stopped - add stop solution to every well before measuring optical density. Technical Limitations Not every raw material or endogenous interference can be comprehensively identified or removed. Results depend on reagent performance, operator technique, and the experimental environment. Use only reagents supplied with this kit and follow the instructions exactly. Incorrect reagent preparation or microplate-reader settings may produce abnormal results. Independent experiments may yield different results even when performed by the same operator; control every step to support reproducibility. Transportation conditions and differences among instruments may cause results to differ from release-testing data. The kit has not been directly compared with other manufacturers' kits or with different methods for the same analyte, so results may not be identical.
Storage	Store the unopened kit at 2-8°C. The unopened kit is stable for 6 months under the specified storage conditions. Refer to the outer packaging label for the exact expiration date and use the kit within the stated shelf life. Do not use expired reagents. After opening, store individual components according to the component-specific conditions listed in the kit-component section.
Reaction conditions	Reagent Preparation 1. Before use, allow all kit components to equilibrate to room temperature for at least 60 minutes. 2. Concentrated Wash Solution: Crystals may form during refrigerated storage; this is normal. Warm in a water bath until the crystals dissolve completely. Dilute the 20× concentrated wash solution 1:20 with distilled water by mixing 1 volume of concentrated wash solution with 19 volumes of distilled water. 3. Substrate Working Solution: Immediately before use, mix Substrate A and Substrate B thoroughly at a 1:1 volume ratio. Use the mixed substrate within 15 minutes. Assay Procedure Bring all reagents and components to room temperature before starting. Duplicate wells are recommended for calibrators, quality-control samples, and test samples. 1. Prepare all required working solutions as described above. 2. Remove the required microplate strips from the foil pouch. Seal the

	remaining strips in the resealable bag and return them to refrigerated storage. 3. Set up calibrator wells, zero wells, blank wells, and sample wells. Add 50 µL of each calibrator concentration to the corresponding calibrator wells. Add 50 µL Sample Diluent to each zero well. Add nothing to the blank wells. Add 50 µL test sample to each sample well. 4. Add 100 µL HRP-labeled detection antibody to every calibrator, zero, and sample well. Do not add it to blank wells. 5. Cover the plate with sealing film and incubate for 60 minutes at 37°C in a water bath or incubator, protected from light. 6. Remove the sealing film and discard the liquid. Tap the plate dry on absorbent paper. Fill each well with wash solution, allow it to stand for 20 seconds, discard the wash solution, and tap the plate dry. Repeat for a total of 5 wash cycles. When using an automatic plate washer, follow the instrument procedure; adding a 30-second soak step may improve precision. After washing and before substrate addition, tap the plate thoroughly dry on clean, lint-free paper. Residual liquid must be completely removed. Proceed immediately to the next step and do not allow the wells to dry. 7. Mix Substrate A and Substrate B at a 1:1 volume ratio. Add 100 µL mixed substrate to every well. Cover the plate with sealing film and incubate for 15 minutes at 37°C in a water bath or incubator, protected from light. 8. Add 50 µL Stop Solution to every well. Read the absorbance of each well at 450 nm.
Unit definition	Result Calculation Use the calibrator concentration as the horizontal axis and the corresponding absorbance value as the vertical axis. Fit the calibrator data with suitable curve-fitting software. A four-parameter logistic model, 4-PL, is recommended, although it is not the only acceptable fitting method. Generate the standard-curve equation and calculate each sample concentration from its absorbance value. If a sample was diluted before testing, multiply the concentration obtained from the standard curve by the dilution factor to obtain the final sample concentration. The operator must establish a standard curve for each assay. Every microplate used in each test must include its own standard curve.

Usage	For Research Use Only. Not for Use in Clinical Diagnosis.

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA