Human IL-6 ELISA Kit

Cat. No. KITG-111 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This kit uses a one-step double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). Samples, standards, and the HRP-labeled detection antibody are added sequentially to microwells pre-coated with an antibody against interleukin 6 (IL-6), followed by incubation and thorough washing. TMB substrate develops a blue color under peroxidase catalysis and changes to yellow after acid is added. Color intensity is positively correlated with the IL-6 concentration in the sample. Absorbance (OD) is measured at 450 nm with a microplate reader, and the sample concentration is calculated.
Species	Human
Conjugation/Label	HRP-labeled detection antibody
Description	Sample Collection, Processing, and Storage Serum: Use pyrogen- and endotoxin-free tubes. Avoid any cell stimulation during handling. After blood collection, centrifuge at 3,000 rpm for 10 minutes and rapidly and carefully separate the serum from the red blood cells. Plasma: Use EDTA, citrate, or heparin as the anticoagulant. Centrifuge at 3,000 rpm for 30 minutes and collect the supernatant. Cell culture supernatant: Centrifuge at 3,000 rpm for 10 minutes to remove particulates and aggregates. Tissue homogenate: Add an appropriate amount of normal saline to the tissue and homogenize. Centrifuge at 3,000 rpm for 10 minutes and collect the supernatant. Sample storage: If samples are not tested promptly after collection, divide them into single-use aliquots and store at -20°C. Avoid repeated freeze-thaw cycles. Thaw at room temperature and ensure that each sample is uniformly and completely thawed

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	before use.
Form	Kit Components Pre-coated microplate: 96-well configuration, 12 wells × 8 strips; 48-well configuration, 12 wells × 4 strips. Standards: 0.3 mL × 6 tubes for either configuration. Sample diluent: 6 mL for the 96-well configuration; 3 mL for the 48-well configuration. Detection antibody-HRP: 10 mL for the 96-well configuration; 5 mL for the 48-well configuration. 20× wash buffer: 25 mL for the 96-well configuration; 15 mL for the 48-well configuration. Dilute as instructed before use. Substrate A: 6 mL for the 96-well configuration; 3 mL for the 48-well configuration. Substrate B: 6 mL for the 96-well configuration; 3 mL for the 48-well configuration. Stop solution: 6 mL for the 96-well configuration; 3 mL for the 48-well configuration. Plate-sealing film: 2 sheets for either configuration. Instruction manual: 1 copy for either configuration. Zip-lock bag: 1 bag for either configuration.
Bio-activity	Kit Performance Accuracy: For linear regression of the standards against their expected concentrations, the correlation coefficient R is ≥ 0.9900. Sensitivity: The lowest detectable concentration is < 1.0 pg/mL. Detection range: 1.0-120 pg/mL. Specificity: No cross-reactivity with other soluble structural analogues. Repeatability: Both intra-assay and inter-assay coefficients of variation are < 15%.
Applications	Quantitative detection of human interleukin 6 (IL-6) in serum, plasma, cell culture supernatants, and tissue homogenates by ELISA.
Notes	Operating Precautions Store the kit at 2-8°C and equilibrate it at room temperature for 20 minutes before use. Crystals may form in the concentrated wash solution; this is normal. Warm it in a water bath until the crystals are completely dissolved before use. Immediately return unused plate strips to the zip-lock bag, seal the bag, and store under cool, dry conditions. The S_0 standard, with a concentration of 0 pg/mL, may be treated as a negative control or blank. When the instructions are followed, the sample is diluted 5-fold; multiply the final result by 5 to obtain the actual

	sample concentration. Strictly follow the stated incubation times, liquid volumes, and order of addition. Mix all liquid components thoroughly before use.
Storage	Store at 2-8°C, protected from light and moisture. Shelf life: 6 months.
Concentration	Standard concentrations S_0-S_5: 0, 7.5, 15, 30, 60, and 120 pg/mL, respectively.
Reconstitution	Dilution of 20× wash buffer: Dilute 1:20 with distilled water by adding 1 part 20× wash buffer to 19 parts distilled water.
Reaction conditions	Plate Washing Manual washing: Empty all liquid from the wells. Fill each well with wash buffer, let stand for 1 minute, discard the wash buffer, and tap the plate dry on absorbent paper. Repeat for a total of 5 washes. Automatic plate washer: Add 350 µ wash buffer to each well, soak for 1 minute, and wash 5 times. Assay Procedure 1. After the foil pouch has equilibrated at room temperature for 20 minutes, remove the required plate strips. Seal the remaining strips in the zip-lock bag and return them to 4°C. 2. Set up standard wells and sample wells. Add 50 µ of a different standard concentration to each standard well. 3. Add 10 µ of the sample to each sample well, followed by 40 µ of sample diluent. Add nothing to the blank well. 4. Except for the blank well, add 100 µ of HRP-labeled detection antibody to every standard and sample well. Seal the wells with plate-sealing film and incubate at 37°C for 60 minutes in a water bath or incubator. 5. Discard the liquid and tap the plate dry on absorbent paper. Fill each well with wash buffer and let stand for 1 minute. Discard the wash buffer and tap dry on absorbent paper. Repeat for a total of 5 washes. A plate washer may also be used. 6. Add 50 µ each of Substrate A and Substrate B to every well. Incubate at 37°C for 15 minutes in the dark. 7. Add 50 µ stop solution to every well. Measure the OD_{450} value of each well within 15 minutes. Result Calculation Create a standard curve in an Excel worksheet with standard concentration on the x-axis and the corresponding OD value on the y-axis. Fit a

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	linear regression curve and calculate each sample concentration from the regression equation. Because the sample is diluted 5-fold during the procedure, multiply the calculated result by 5 to obtain the actual sample concentration.
Usage	For research use only. Not for medical diagnosis, clinical experiments, or human experiments. The experimenter bears all consequences of use outside these restrictions. Strictly follow the instructions in this manual. The experimenter bears the consequences of deviations from the instructions. Required Materials and Equipment Microplate reader capable of measurement at 450 nm. High-precision pipettes and tips covering 0.5-10 µ, 2-20 µ, 20-200 µ, and 200-1,000 µ. 37°C incubator.

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