

Human IL-6 ELISA Kit

Catalog nos. KHC0061
KHC0062
KHC0061C

Pub. No. MAN0014630 Rev 1.0


Description

The Human Interleukin-6 (Human IL-6) ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) is designed to detect and quantify the level of Human IL-6 in human serum, plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Human IL-6.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2°C to 8°C.

Components	Cat. no. KHC0061 96 tests	Cat. no. KHC0062 192 tests	Cat. no. KHC0061C 480 tests
Human IL-6 Antibody Coated Wells. 96 well plate.	1 plate	2 plates	5 plates
Human IL-6 Biotin Conjugate. Contains 7.7 mM sodium azide.	6 mL	2 × 6 mL	5 × 6 mL
Human IL-6 Standard, recombinant Human IL-6. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials	10 vials
Wash Buffer Concentrate (25X).	100 mL	100 mL	2 × 100 mL
Standard Diluent Buffer. Contains 7.7 mM sodium azide.	25 mL	2 × 25 mL	5 × 25 mL
Streptavidin-HRP (100X). Contains 0.04% ProClin™ 300, 3.3 mM thymol, and 0.06% methoxyphenol.	0.125 mL	2 × 0.125 mL	5 × 0.125 mL
Streptavidin-HRP Diluent. Contains 0.04% ProClin 300.	25 mL	25 mL	3 × 25 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB).	25 mL	25 mL	3 × 25 mL
Stop Solution.	25 mL	25 mL	3 × 25 mL
Adhesive Plate Covers.	3	6	15

 **CAUTION!** This kit contains materials with small quantities of sodium azide and ProClin 300. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. ProClin 300 is toxic. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Materials required but not provided

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer—automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

General Guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at thermofisher.com/techresources for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

For Research Use Only. Not for use in diagnostic procedures.

Manufacturing Site • 7335 Executive Way • Frederick • MD 21704 • E-mail: techsupport@lifetech.com

Prepare 1X Wash Buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
3. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

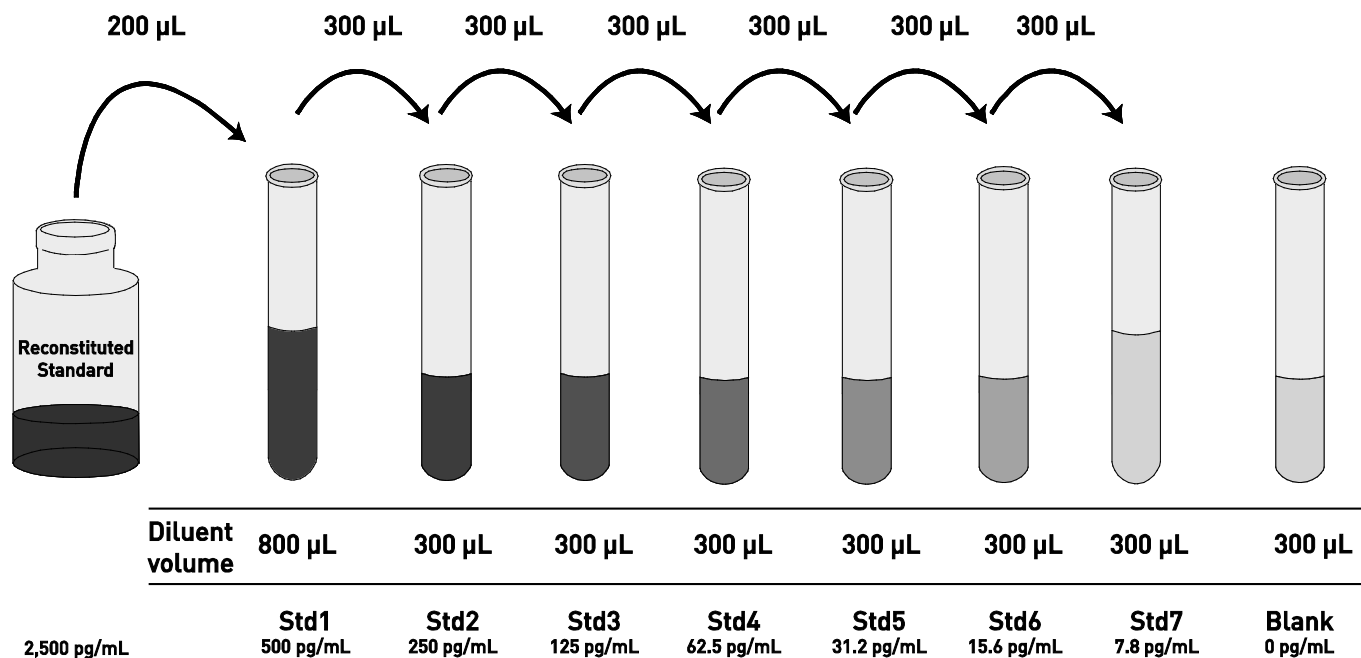
Dilute samples

- Samples > 500 pg/mL should be diluted with Standard Diluent Buffer.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Human IL-6 Standard to 2,500 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 2,500 pg/mL Human IL-6. **Use the standard within 1 hour of reconstitution.**
2. Add 200 μ L Reconstituted Standard to one tube containing 800 μ L Standard Diluent Buffer and label as 500 pg/mL Human IL-6.
3. Add 300 μ L Standard Diluent Buffer to each of 7 tubes labeled as follows: 250, 125, 62.5, 31.2, 15.6, 7.8, and 0 pg/mL Human IL-6.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP solution within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

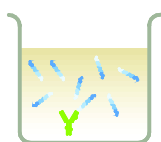
1. Allow Streptavidin-HRP (100X) to reach room temperature and mix gently.
2. For each 8-well strip used in the assay, pipet 10 μ L Streptavidin-HRP (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
3. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 3 hours.**

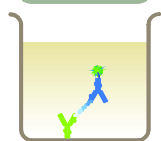
IMPORTANT! Perform a standard curve with each assay.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.



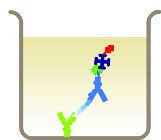
Bind antigen

1. Add 100 µL Standard Diluent Buffer to zero wells except the chromogen blanks.
2. Add 100 µL of standards or samples to the appropriate wells.



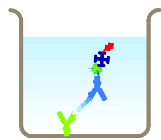
Add Biotin Conjugate

3. Add 50 µL Human IL-6 Biotin Conjugate solution into each well except the chromogen blanks.
4. Tap the side of the plate to mix. Cover the plate with plate cover and incubate for 2 hours at room temperature.
5. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.



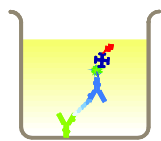
Add Streptavidin-HRP

6. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
7. Cover the plate with the plate cover and incubate for 30 minutes at room temperature.
8. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.



Add chromogen

9. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
10. Cover the plate with the plate cover and incubate for 30 minutes at room temperature **in the dark**.
Note: TMB should not touch aluminum foil or other metals.



Add stop solution

11. Add 100 µL of Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.



Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

Note: Dilute samples producing signals greater than that of the highest standard in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0-500 pg/mL Human IL-6.

Standard Human IL-6 (pg/mL)	Optical Density (450 nm)
500	2.59
250	1.33
125	0.73
62.5	0.40
31.2	0.24
15.6	0.13
7.8	0.10
0	0.06

Specificity

Buffered solutions of a panel of substances at 50 ng/mL were assayed with the Human IL-6 kit. The following substances were tested and found to have no cross-reactivity: **Human** IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-7, IL-8, IL-10, G-CSF, GM-CSF, IFN- α , IFN- γ , LIF, MIP-1 α , MIP-1 β , MCP-1, OSM, RANTES TGF- β , TNF- α , TNF- β ; **mouse** IL-6; **rat** IL-6.

Sensitivity

The minimum detectable dose of Human IL-6 is <2 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 32 times, and calculating the corresponding concentration.

Performance characteristics, continued

Intra-assay precision

Samples of known Human IL-6 concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	38.8	101.2	242.7
SD	3.0	5.8	12.5
%CV	7.7	5.7	5.1

SD = Standard Deviation; CV = Coefficient of Variation

Recovery

The recovery of Human IL-6 added to normal human serum and normal human plasma averaged 88% and 70%, respectively. The recovery of Human IL-6 added to tissue culture medium containing 10% fetal bovine serum averaged 111%.

Expected values

Eight samples, that consisted of 10% human whole blood in culture medium stimulated with LPS/PHA, were assayed for Human IL-6. The values ranged from 720-12,000 pg/mL. The mean value was 2,500 pg/mL.

Inter-assay precision

Samples were assayed 8 times in 5 different assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	35.3	97.4	236.7
SD	3.3	6.4	18.5
%CV	9.3	6.5	7.8

SD = Standard Deviation; CV = Coefficient of Variation

Linearity of dilution

Human serum containing 222 pg/mL of measured Human IL-6 was serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded an average correlation coefficient of 0.998.








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Product label explanation of symbols and warnings

 REF	Catalog Number	 LOT	Batch code	 Temperature limitation	 Use by	 Manufacturer	 Consult instructions for use	 Caution, consult accompanying documents
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