Human IL-6 ELISA Kit

Catalog Number KAC1261 (96 tests)

Pub. No. MAN0018799 Rev. B.0

CAUTION! This kit contains materials with small quantities of sodium azide. 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. 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.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Human IL-6 ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human IL-6 in serum, plasma, cell culture supernatant, and other biological fluids. The assay recognizes both natural and recombinant human IL-6.

Interleukin-6 (IL-6) is a 184 amino acid polypeptide produced by T- and B-cells, monocytes, fibroblasts, keratinocytes, endothelial cells, mesangial cells, astrocytes, and bone marrow stroma cells. It regulates the growth and differentiation of various cell types and plays a role in hematopoiesis and inflammation.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C. Store the Wash Solution Concentrate at room temperature. When stored as indicated, all reagents are stable until the expiration date.

Contents	Cat. No. KAC1261 (96 tests)
Specimen diluent, human plasma with preservatives for cell culture or urine	2 vials
Incubation buffer, buffer with preservatives for serum or plasma	11 mL
Standards 0 to 5 in human plasma with preservatives; lyophilized. Refer to vial label for quantity and reconstitution volume.	6 vials
Controls 1 and 2 in human plasma with preservatives; lyophilized. Refer to vial label for reconstitution volume and range.	2 vials
IL-6 Antibody-Coated Wells, 96-well strip-well plate	1 plate
Anti-IL-6-HRP Conjugate, in a buffered solution with proteins and preservatives	11 mL
Wash Solution Concentrate (200X)	10 mL
Chromogenic TMB (tetramethylbenzidine) in DMF	25 mL
Stop Solution (1 N HCl)	12 mL

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at 450 nm, 490 nm, and 650 nm (polychromatic reading)
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Horizontal microplate shaker capable of 700 rpm ± 100 rpm
- Magnetic stirrer

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 2 mL of Wash Solution Concentrate (200X) with 398 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Use a magnetic stirrer to mix the solution.

   Note: Use 1X Wash Buffer on the same day it is prepared. Discard unused 1X Wash Buffer at the end of the day.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- 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.

Reconstitute standards

Note: Standards are stable for 4 days at 2–8°C. For longer term storage, make aliquots and store at –20°C for up to 2 months. Avoid successive freeze thaw cycles.

Reconstitute Standards 0 to 5 by adding 1 mL of distilled water to each vial.

Note: The standards are used to create a standard curve. 1 pg of calibrator is equivalent to 5 mIU NIBSC 1st RR 93/722. See the exact values of each standard on vial labels.

Reconstitute Controls

Note: Controls are stable for 4 days at 2–8°C. For longer term storage, make aliquots and store at –20°C for up to 2 months. Avoid successive freeze thaw cycles.

Reconstitute Controls 1 and 2 by adding 1 mL of distilled water to each vial.

If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy can be determined.

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

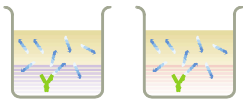
- Dilute the sample as needed:
  - **a.** For serum and plasma samples, dilute with reconstituted Specimen diluent.
  - **b.** For cell culture supernatant and urine samples, dilute with Incubation buffer or the type of culture medium used to grow the cells.
- If samples generate values higher than the highest standard, dilute samples further and repeat the assay.

Perform ELISA (Total assay time: 2.5 hours)

IMPORTANT! Perform a standard curve with each assay.

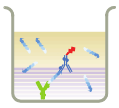
- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen



1.1. Pipet 50 µL of Incubation buffer into the appropriate wells for the Standards and Controls.
1.2. Pipet 50 µL of Incubation buffer into the appropriate wells for serum/plasma samples or pipet 50 µL of Specimen diluent into the appropriate wells for cell culture supernatant/urine samples.
1.3. Add 100 µL of standards, controls, or samples (see “Pre-dilute samples” on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
1.4. Incubate for 1 hour at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm.
1.5. Thoroughly aspirate the solution and wash wells 3 times with 1X Wash Buffer.

2 Add HRP Conjugate solution



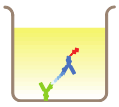
2.1. Add 100 µL of anti-IL-6 conjugate into all the wells.
2.2. Add 50 µL of Specimen diluent into each well except the chromogen blanks.
2.3. Incubate for 1 hour at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm.700 rpm ± 100 rpm.
2.4. Thoroughly aspirate the solution from the wells and wash wells 3 times with 1X Wash Buffer.

3 Add Chromogenic TMB

3.1. Add 200 µL of Chromogenic TMB to each well. The substrate solution begins to turn blue.
3.2. Incubate for 15 minutes at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm in the dark.

Note: TMB should not touch aluminum foil or other metals.

4 Add Stop Solution



Add 100 µL Stop Solution to each well. Tap the side of the plate 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 3 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 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 the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data obtained for standards 0 to 5 are for illustration only and should never be used in place of a real time standard curve.

Standard	Concentration (pg/mL)	Optical Density (450 nm)
5	1690	3.88
4	462	2.24
3	147	0.74
2	45	0.24
1	16	0.10
0	0	0.04

Inter-assay precision

Samples were assayed in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2
Mean (pg/mL)	70.7	194.9
Standard Deviation	5.3	4.3
% Coefficient of Variation	7.5	2.2

Intra-assay precision

Samples of human IL-6 were assayed in multiple assays to determine precision within an assay.

Parameters	Sample 1	Sample 2
Mean (pg/mL)	75.6	205.4
Standard Deviation	6.1	10.1
% Coefficient of Variation	5.6	4.7

Expected values

These values are given only for guidance and it is recommended that each laboratory establishes its own normal values.

The results of 80 serum samples from apparently healthy persons, 49 samples had non-detectable levels of IL-6, 29 had low but detectable levels of IL-6 (range: 3.0-8.5 pg/mL), one sample showed 24.5 pg/mL, and another showed 72.3 pg/mL.

Interference

To ensure the absence of any interference by the soluble receptors sIL-6 receptor and sgp-130 on the assay, a recovery was performed in the presence of high concentration of sIL-6R, sgp-130, and a mix of the two receptors. The results shown in the following table indicate that now interference was observed.

IL-6 (pg/mL)	Measurement with 100 ng/mL sIL-6 (pg/mL)	Measurement with 100 ng/mL sgp-130 (pg/mL)	Measurement with 100 ng/mL each of sIL-6 and sgp-130 (pg/mL)
0	0	0	0
207	209	202	203
846	875	850	869
1766	1890	1766	1732

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 20 times, and calculating the corresponding concentration.

Specificity

No significant cross-reaction was observed in presence of 50 ng of IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-7, IL-8, IL-10, GM-CSF, TNF-α, TNF-β, IFN-α, IFN-γ, TGF-β, OSM, MIP-1α, MIP-1β, LIF, MCP-1, and RANTES. A very tenuous cross-reaction (0.06%) is observed with G-CSF. This kit is specific for natural and recombinant Hu IL-6.

Recovery

Sample	Added IL-6 (pg/mL)	Recovery IL-6 (pg/mL)	Recovery %
Serum 1	1066	1035	97.1
	547	541	98.9
	228	234	102.6
Serum 2	1066	1110	104.1
	547	531	97.1
	228	250	109.7
Plasma 1	804	812	101
	409	419	102
	214	211	99
Plasma 2	804	813	101
	409	407	100
	214	185	86
Cell Culture Medium	772	709	91
	347	384	109
	166	188	110

Linearity of dilution

Dilution	Serum		Plasma	
	Measured conc. (pg/mL)	Theor. conc. (pg/mL)	Measured conc. (pg/mL)	Theor. conc. (pg/mL)
1/1	—	966	—	835.9
1/2	478	483	411.9	418
1/4	247	241.5	212.2	209
1/8	130	120.8	106.7	104.5
1/16	54.1	60.4	47.9	52.2
1/32	23.3	30	—	

Dilution	Cell culture medium	
	Measured conc. (pg/mL)	Theor. conc. (pg/mL)
1/1	—	763
1/2	363	381.5
1/4	178	190.8
1/8	87	95.4
1/16	41	47.7

Limited product warranty

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Products manufactured at this site:

- KAC1261

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

The information in this guide is subject to change without notice.