Human IL-10 ELISA Kit

Catalog Number KHC0101 (96 tests), KHC0102 (2 × 96 tests), KHC0101C (5 × 96 tests)

Pub. No. MAN0003760 **Rev.** 3.0 (30)

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-10 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-10 in human serum, plasma (EDTA, heparin), buffered solution and cell culture medium. The assay recognizes both natural and recombinant human IL-10.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KHC0101 (96 tests)
Hu IL-10 Standard; lyophilized.	2 vials
Standard Diluent Buffer; contains 8 mM sodium azide	25 mL
Incubation Buffer; contains 8 mM sodium azide	25 mL
Antibody-Coated Wells, 96-well plate	1 plate
Hu IL-10 Biotin Conjugate; contains 8 mM sodium azide	11 mL
Streptavidin-Peroxidase (HRP), (100X); contains 1.3 mM thymol	0.125 mL
Streptavidin-Peroxidase (HRP) Diluent; contains 1.3 mM thymol and 0.05% Proclin™ 300	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, Adhesive strips	4

Materials required but not supplied

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

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 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

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.

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.

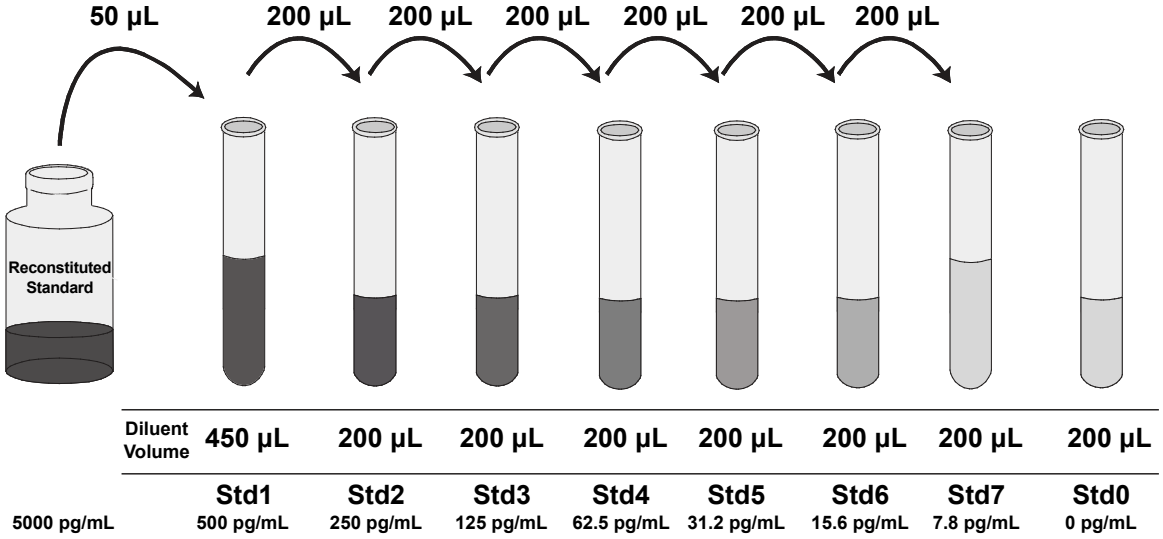
Perform sample dilutions with Standard Diluent Buffer (serum/plasma) or with the corresponding cell culture medium (cell culture supernatant).

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: This assay has been calibrated against the International Standard preparation (92/516) for human IL-10 (NIBSC, Hertfordshire, UK, EN6 3QG). One microgram equals 13,200 International Units.

1. Reconstitute Hu IL-10 Standard to 5,000 pg/mL with Standard Dilution 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 5,000 pg/mL human IL-10. **Use the standard within 1 hour of reconstitution.**
2. Add 50 µL Reconstituted Standard to one tube containing 450 µL Standard Diluent Buffer and mix. Label as 500 pg/mL human IL-10.
3. Add 200 µL 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-10.
4. Make serial dilutions of the standard as shown in the following 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 within 15 minutes of usage.

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

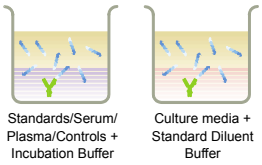
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP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

Perform ELISA (Total assay time: 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



a. Add 50 µL of **Incubation Buffer** to wells for serum or plasma samples, and standards; or 50 µL of **Standard Diluent Buffer** to the wells for cell culture samples. Leave the wells for chromogen blanks empty.
b. Add 50 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
c. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

a. Add 100 µL Hu IL-10 Biotin Conjugate solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 2 hours at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 Add Streptavidin-HRP

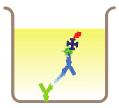
a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

4 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

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

5 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 2 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 (serum/plasma) or with the corresponding cell culture medium (cell culture supernatant) 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-10.

Standard Human IL-10 (pg/mL)	Optical Density (450 nm)
500	2.84
250	1.68
125	0.99
62.5	0.56
31.2	0.35
15.6	0.19
7.8	0.11
0	0.03

Inter-assay precision

Samples were assayed 24 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	55.7	140.3	289.4
Standard Deviation	1.6	3.9	16.0
% Coefficient of Variation	2.9	2.8	5.5

Intra-assay precision

Samples of known human IL-10 concentration were assayed in replicates of 24 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	57.6	138.0	333.3
Standard Deviation	1.7	4.0	15.9
% Coefficient of Variation	2.9	2.9	4.8

Expected values

Each laboratory must establish its own normal values. For guidance, the mean of 20 normal human plasma and sera was <1 pg/mL.

Cell culture supernatants from human whole blood (WB) cells cultured in RPMI supplemented with 5% FCS for 24, 48 or 72 hours under different stimulation conditions were evaluated using the Human IL-10 ELISA Kit.

Stimulation condition	Human IL-10 (pg/mL)		
	24 hr	48 hr	72 hr
None	0	0	0
LPS (25 µg/mL) + PHA (5 µg/mL)	223	412	250
PMA (100 ng/mL) + ionomycin (100 ng/mL)	35	21	10
LPS (25 µg/mL)	211	125	45

Linearity of dilution

Cell culture or serum samples containing human IL-10 were serially diluted in corresponding medium or Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Dilution	Cell culture medium			Serum		
	Measured (pg/mL)	Expected		Measured (pg/mL)	Expected	
		(pg/mL)	%		(pg/mL)	%
Neat	412	—	—	468	—	—
1/2	215	206	104	229	234	98
1/4	109	103	106	115	117	98
1/8	54	52	104	56	59	95
1/16	26	26	100	28	30	93

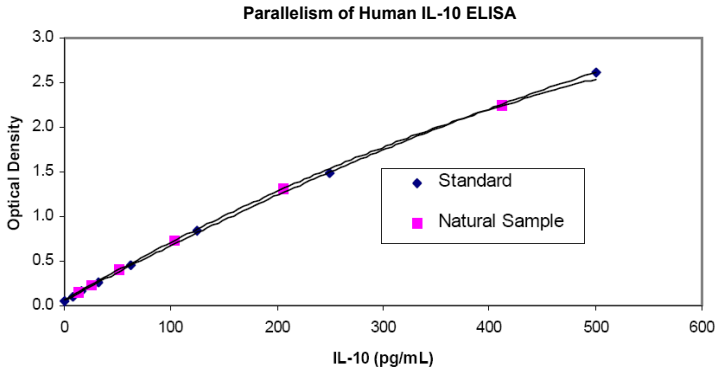
Recovery

The recovery of human IL-10 added to human serum or plasma (EDTA, heparin) and tissue culture medium containing fetal bovine serum (FBS) was measured using the Human IL-10 ELISA Kit.

Sample	Average % Recovery
Serum or plasma	93
Tissue culture medium + 10% FBS	99

Parallelism

Natural human IL-10 was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the standard curve. Parallelism demonstrates that the standard accurately reflects the human IL-10 content in samples.



Sensitivity

The analytical sensitivity of the assay is <1 pg/mL human IL-10. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times.

Specificity

Buffered solutions of a panel of substances at 50 ng/mL were assayed with the Human IL-10 ELISA Kit. The following substances were tested and found to have no cross-reactivity: **human** IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, TNF-α, TNF-β, IFN-α, IFN-β, IFN-γ, TGF-β, GM-CSF, OSM, MIP-1α, MIP-1β, LIF, MCP-1, G-CSF, RANTES, GRO, IP-10 and SCF. This human IL-10 assay is specific for human natural and recombinant human IL-10.

Cross-reactivity

Low level (<0.2 %) cross-reactivity was observed with BCRF1 (Epstein-Barr Virus IL-10).

Limited product warranty

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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

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

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