Performance characteristics, continued

Intra-assay precision

Samples with known Human IL-23 concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	119.3	432.5	1837.0
SD	6.7	20.4	88.5
%CV	5.6	4.7	4.8

SD = Standard Deviation; CV = Coefficient of Variation

Inter-assay precision

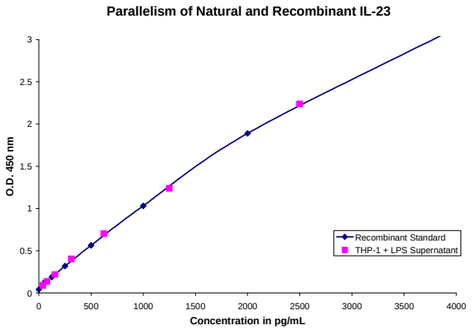
Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	117.9	444.8	1836.2
SD	8.1	19.8	82.4
%CV	6.8	4.5	4.5

SD = Standard Deviation; CV = Coefficient of Variation

Parallelism

Natural Human IL-23 from THP-1 Cells stimulated with 1 µg/mL LPS was serially diluted in Standard Diluent Buffer. The optical density of the expected value of each dilution was plotted against the Human IL-23 standard curve. Parallelism was demonstrated by the figure below and indicated that the standard accurately reflects Human IL-23 content in samples.



Linearity of dilution

Tissue culture media and serum spiked with recombinant Human IL-23 was serially diluted in 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 for both sample types.

	Serum			Tissue culture Media		
Dilution	Measured	Expected	% Expected	Measured	Expected	% Expected
1/2	1852.1	1852.1	—	1780.6	1780.6	—
1/4	864.1	926.0	93.3	908.5	890.3	102.0
1/8	412.6	463.0	89.1	453.7	445.1	101.9
1/16	223.1	231.5	96.4	263.8	222.6	118.5
1/32	94.8	115.8	81.9	113.9	111.3	102.3

Recovery

Fixed quantities of recombinant Human IL-23 were spiked into human serum and various tissue culture media containing 1% or 10% fetal bovine serum. Recoveries were measured on the Human IL-23 ELISA.

Sample type	Range	Average % Recovery
Human serum*	78.6–116.2	96.9
DMEM and 1% FBS*	76.9–89.7	82.9
DMEM and 10% FBS*	84.8–93.4	89.3
RPMI and 1% FBS*	74.0–97.0	90.3

*All samples were pre–diluted 2–fold as described in sample preparation procedure. Plasma recoveries are <80%.

High-dose hook effect

No hook effect was observed with concentrations up to 1 µg/mL.

Important licensing information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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

novex
life technologies

Human IL-23 ELISA Kit

Catalog no. KHC0231 **Quantity:** 96 tests

Pub. No. MAN0003322 **Rev** 2.0

Description

The Human IL-23 ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of natural and recombinant Human Interleukin-23 (Human IL-23) in serum, buffered solution, and tissue culture medium.

IL-23 is a cytokine that plays a role in the maturation of memory T-cells. Like the closely related cytokines IL-12 and IL-27, IL-23 is a disulfide-linked heterodimer of approximately 70kDa. The two subunits of IL-23 are designated p19 and p40. A search of protein sequence databases for proteins bearing homology with IL-6 led to the discovery of the p19 subunit. In addition to IL-6, the p19 subunit also bears homology with G-CSF and the p35 subunit of IL-12. The p40 subunit, which is common to both IL-23 and IL-12, shares homology with the receptors for IL-6 and ciliary neurotrophic factor. In humans, the p19 subunit maps to 12q13, while the p40 subunit maps to 5q31. The expression of the p19 and the p40 subunits is differentially regulated. The orchestrated production of both subunits is required for the production of the biologically active cytokine.

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	96 Test Kit
Human IL-23 Standard (recombinant Human IL-23), lyophilized, contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
Standard Diluent Buffer, contains 0.1% sodium azide	25 mL
Antibody Coated Wells, 96 Wells per plate.	1 plate
Human IL-23 Biotin Conjugate, (Biotin-labeled anti-IL-23), contains 0.1% sodium azide	11 mL
Streptavidin-HRP (100X), contains 3.3 mM thymol	0.125 mL
Streptavidin HRP Diluent, contains 3.3 mM thymol	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

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.

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 **www.lifetechnologies.com/manuals** 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 the 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

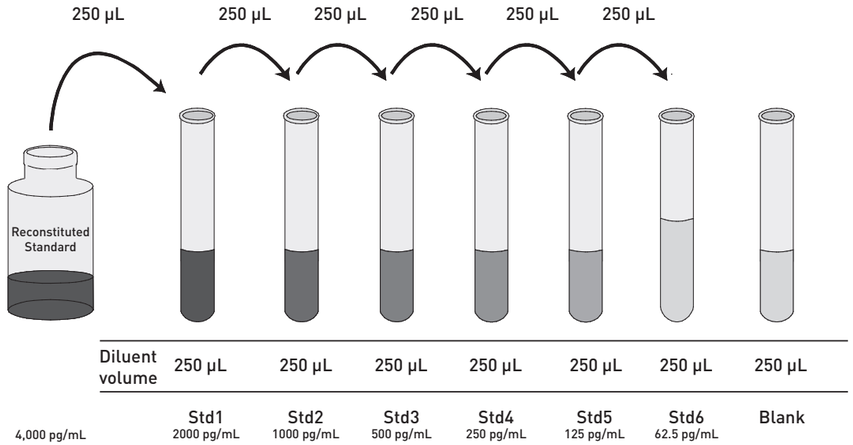
- Dilute **serum and plasma** samples 2-fold in Standard Diluent Buffer.
- Buffered solutions, cell culture samples, and controls may be assayed without dilution.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Dilute standards

Note: Plastic tubes must be used for diluting standards. **Do not use glass.**

1. Reconstitute IL-23 Standard to 4,000 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. Use the Standard within 1 hour of reconstitution. Label as 4,000 pg/mL IL-23.
2. Add 250 µL Standard Diluent Buffer to each of 6 tubes labeled as follows: 2000, 1000, 500, 250, 125, and 62.5 pg/mL of Human IL-23.

   Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps. Discard any remaining reconstituted standard or freeze at -80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity. Return the Standard Diluent Buffer to the refrigerator.



Prepare Streptavidin-HRP solution

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

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

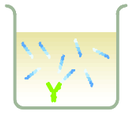
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 990 µL of HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) 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 4 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

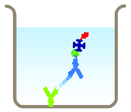
1. Add 100 µL of standards or controls to the appropriate wells. For serum or tissue culture supernatant, add 75 µL of Standard Diluent Buffer followed by 75 µL of sample to a polypropylene tube. Mix the buffered sample well and add 100 µL to the appropriate well.
2. Cover the plate with plate cover and incubate for 2 hours at room temperature.
3. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add detector antibody

4. Add 100 µL Human IL-23 Biotin Conjugate solution into each well except chromogen blanks.
5. Cover the plate with plate cover and incubate for 1 hour at room temperature.
6. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

Add Streptavidin-HRP

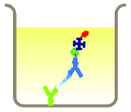
7. Add 100 µL Streptavidin-HRP (see page 2) into each well except the chromogen blanks.
8. Cover the plate with plate cover and incubate for 30 minutes at room temperature.
9. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.



Add chromogen

10. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
11. Cover the plate with 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

12. Add 100 µL 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 0–4000 pg/mL Hu IL-23.

Standard Hu IL-23 (pg/mL)	Optical Density (450 nm)
4,000	3.081
2,000	2.022
1,000	1.167
500	0.634
250	0.354
125	0.191
63	0.113
0	0.032

Specificity

Buffered solutions of a panel of substances ranging in concentrations from 3000–45,000 pg/mL were assayed with the Human IL-23 kit and found to have no cross–reactivity: human IL-1α, IL-1β, IL-2, IL-5, IL-6, IL-7, IFN-α, IFN-γ, GM-CSF, IL-13, IL-15, IL-17, MIP-1β, eotaxin, RANTES, IL-3, IL-4, IL-8, IL-10, TNF-α, IL-12, MCP-1, MIP-1α, IP-10, and MIG; mouse FGF, GM-CSF, IL1-β, IL-2, IL-6, IL-10, IL-17, IP-10, MIG, MIP-1α, IFN-γ, IL-1α, IL-4, IL-5, IL-12, IL-13, KC, MCP-1, TNF-α, and VEGF.

Sensitivity

The minimum detectable concentration of Human IL-23 is <20 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.