Human IL-23 ELISA Kit

Catalog Number：CEA-C093

Assay Tests: 96 tests

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

IMPORTANT: Please carefully read this user guide before performing your experiment.

Product information

This kit is specifically designed for the accurate quantitation of human IL-23 from cell culture supernates, serum and plasma.

The principle of this assay employs a quantitative sandwich enzyme immunoassay approach. Initially, a microplate is coated with a capture antibody. Then, samples and biotinylated capture antibody are added to the wells. After the removal of any unbound materials through washing, streptavidin-HRP (SA-HRP) conjugate is added to the wells. Streptavidin has a very high affinity for biotin, so it binds to the biotinylated capture antibody that is already bound to the target antigen. After washing, a substrate specific to HRP is added to the wells. HRP catalyzes a reaction that converts the substrate into a detectable signal, often a color change or luminescence, depending on the substrate used. This enzymatic reaction amplifies the signal, allowing for higher sensitivity in detecting the target analyte. The intensity of the signal is measured using a spectrophotometer.

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NOTE:

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Please do not use the kit after the expiration date indicated on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.

Manufactured and distributed by
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Contents

The kit contains sufficient reagents for 96 wells.

Catalog	Contents	Amount
CEA093-C01	Pre-coated Anti-IL-23 Antibody Microplate	1 plate
CEA093-C02	Human IL-23 Standard	500 ng/mL×2
CEA093-C03	Biotin-Anti-IL-23 Antibody Con. Solution	100 µL
CEA093-C04	Biotin-Antibody Dilution Buffer	15 mL
CEA093-C05	Streptavidin-HRP Con. Solution	500 µL
CEA093-C06	Streptavidin-HRP Dilution Buffer	15 mL
CEA093-C07	20× Washing Buffer	50 mL
CEA093-C08	Sample Dilution Buffer	15 mL×2
CEA093-C09	Substrate Solution	12 mL
CEA093-C10	Stop Solution	6 mL

NOTE: Bubbles in microplate wells do not affect the experiment and require no action. Proceed with the experimental procedures and methods described below.

Storage

Keep the unopened kit stored at 2-8 °C. Avoid using the kit beyond its expiration date.
For opened kit and reconstituted reagents, with the exception of the two contents listed in following table, others can be stored for up to 30 days at 2-8 °C.

Contents	Storage conditions
Pre-coated Anti-IL-23 Antibody Microplate	Return unused wells to the foil pouch, reseal along entire edge. May be stored for up to 1 month at 2-8°C.
Human IL-23 Standard	**Limit to one freeze-thaw cycle.**

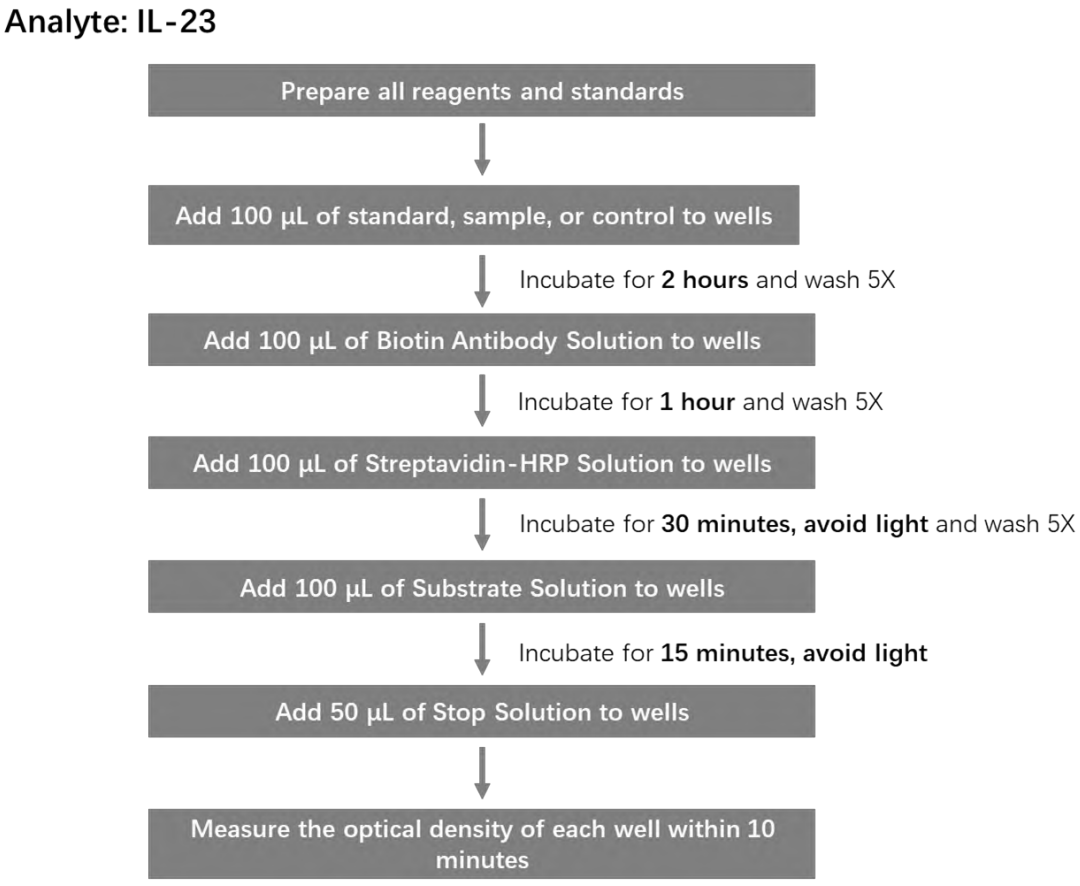
NOTE: Streptavidin-HRP Con. Solution and Substrate Solution should avoid light.

IMPORTANT: Bring all reagents to room temperature before use. If crystals have formed in buffer solution, place the buffer solution in a 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

Required materials not supplied.

Instrument	Microplate reader capable of measuring absorbance at 450 nm
Reagents	Deionized, ultrapure or distilled water
Consumables	50 mL and 500 mL graduated cylinders
	Pipettes and pipette tips
	Tubes to prepare standard dilutions.

Workflow



NOTE: Incubation temperature is 18 ℃-25 ℃

Prepare the working buffers and standard dilutions.

Prepare the working buffers.

1. 1×Washing Buffer: Dilute 50 mL 20×Washing Buffer with deionized or distilled water to 1000 mL.
2. Biotin-Anti-IL-23 Antibody Solution: Add 60 µL of Biotin-Anti-IL-23 Antibody Con. Solution to 12 mL Biotin-Antibody Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.
3. Streptavidin-HRP Solution: Add 300 µL of Streptavidin-HRP Con. Solution to 12 mL of Streptavidin-HRP Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.

Prepare the reconstituted standard.

Reconstitute the provided lyophilized product（CEA093-C02） with deionized or distilled water, dissolve at room temperature for 15-30 minutes, and mix by gently pipetting. (Refer to the vial label for reconstitution volume.) The concentration of reconstituted human IL-23 Standard is 500 ng /mL.

NOTE: *Avoiding vigorous shaking or vortexing.* ***Limit to one freeze-thaw cycle.***

Prepare the standard serial dilutions.

1. Label a tube "**Cm**". Add 12 µL of the reconstituted human IL-23 Standard and 613 µL of Sample Dilution Buffer to tube Cm, gently mix well.
2. Label 7 tubes, one for each standard point: Std.-1, Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7.
3. Add 50 µL of the liquid from **Cm** and 950 µL of Sample Dilution Buffer to tube Std.-1, thoroughly mix (Std.-1 =480 pg/mL).
4. Prepare 1:1 serial dilution for the standard curve as follows: Add 500 µL of Sample Dilution Buffer to each tube (Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7).
5. Transfer 500 µL of liquid from Std.-1 to the tube Std.-2, and thoroughly mix (Std.-2 = 240 pg/mL).
6. Continue to transfer 500 µL of liquid from previous dilution tube to the next dilution tube until add liquid to tube Std.-7.
7. Sample Dilution Buffer serves as zero standard (blank).

Prepare the specimen

To reduce matrix interference of samples, it is necessary to determine the MRD (Minimum Required Dilution) of the samples. **For serum and plasma samples from humans, an MRD of 5 is recommended as a reference**. The specific MRD validation can be initiated at an MRD of 2.5. Based on the target dilution, the dilution factor can be increased stepwise and the accuracy of the results analyzed to identify the optimal dilution factor.

PROCEDURE OF ASSAY

1. Add 100 µL of IL-23 Standard, sample, or control to wells. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **2 hours**.
2. Aspirate each well and add 300 µL of 1×Washing Buffer to each well, gently tap the plate for **1 minute**. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. Repeat the wash process four times for a total of five washes.
3. Add 100 µL Biotin-Anti-IL-23 Antibody Solution to each well, Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hour.**
4. Repeat step 2.
5. Add 100 µL of Streptavidin-HRP Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **30 minutes, avoid light.**
6. Repeat step 2.
7. Add 100 µL of Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **15 minutes, avoid light**.
8. Add 50 µL of Stop Solution to each well. Tap the plate gently to ensure thorough mixing. ***Note:*** *the color in the wells should change from blue to yellow.*
9. Read the absorbance at 450nm and 630nm using Microplate reader within 10minutes.

   Note: *To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

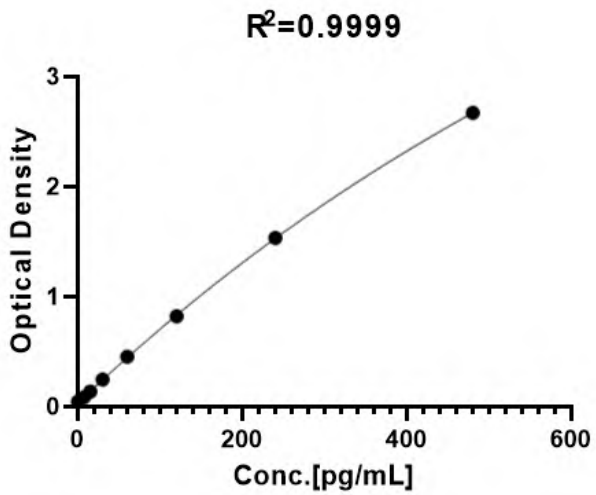
CALCULATION OF RESULTS

1. Compute the average of the duplicated readings for every standard, control, and sample.
2. Establish a standard curve by processing the data using computer software capable of executing a **four-parameter logistic (4-PL)** curve fitting.
3. Normal range of Standard curve: $R^2 \geqslant 0.9900$.
4. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.

Typical data

Note: *For each experiment, a standard curve needs to be set for each microplate, and the specific OD value may vary depending on different laboratories, testers, or equipment. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.*

IL-23 Standard (pg/mL)	$OD_{450nm-630nm}$
480	2.673
240	1.535
120	0.824
60	0.457
30	0.248
15	0.138
7.5	0.086
Blank	0.048



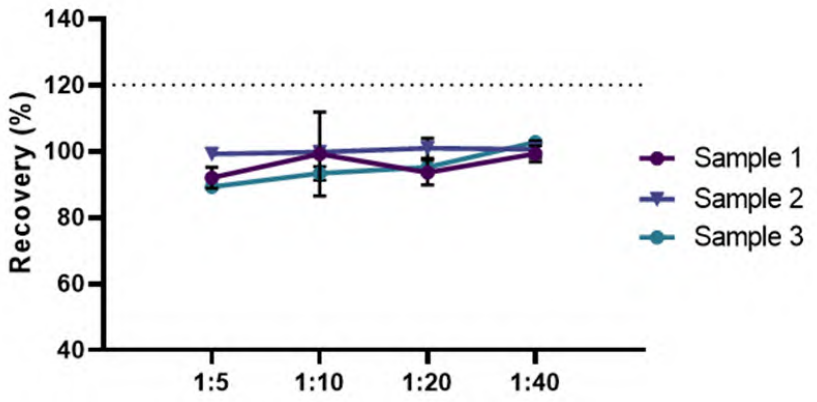
PERFORMANCE CHARACTERISTICS

1. Sensitivity

The minimum detectable concentration (MDC) of IL-23 is typically less than 6 pg/mL. The MDC was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

2. Linearity

Three samples (Serum) spiked with high concentrations of IL-23 were serially diluted with dilution buffer to produce samples with values within the dynamic range of the assay and then assayed. The average recovery of IL-23 for serum samples is 97.11%.



3. Intra-Assay Precision

Eight replicates of each of 3 samples containing different IL-23 concentrations were tested in one assay. Acceptable criteria: CV < 10%.

Sample Concentration (pg/mL)	Mean (pg/mL)	SD	Numbers	CV (%)
360	333.076	16.308	8	4.9
60	57.576	3.443	8	6.0
22.5	21.507	1.250	8	5.8

4. Inter-Assay Precision

Three samples containing different concentrations of IL-23 were tested in independent assays. Acceptable criteria: CV<15%.

Sample Concentration (pg/mL)	Mean (pg/mL)	SD	Numbers	CV (%)
360	332.763	16.634	24	5.0
60	58.845	3.141	24	5.3
22.5	22.252	1.432	24	6.4

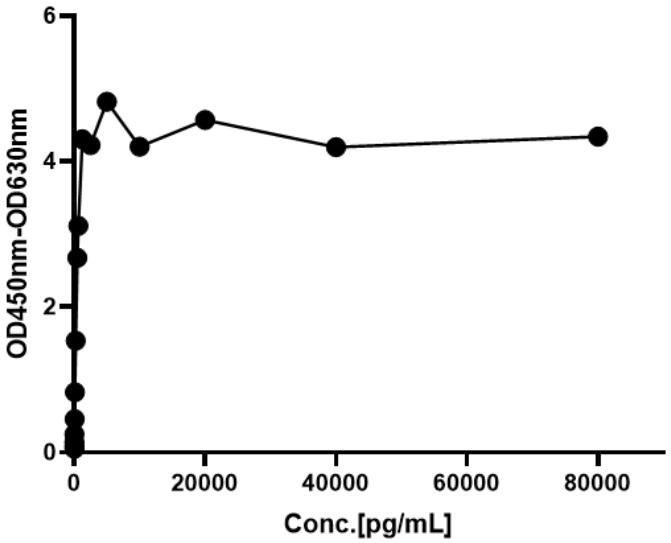
5. Recovery

Recombinant IL-23 was spiked into 3 human serum samples, and then analyzed. The average recovery of IL-23 for serum samples is 94.68%.

Sample ID	Conc Measured (pg/mL)	Conc Added (pg/mL)	Conc Recovered (pg/mL)	Recovery (%)
1	351.578	360	351.578	97.66
	57.151	60	57.151	95.25
	20.643	22.5	20.643	91.75
	0.000	--	--	--
2	310.689	360	310.689	86.30
	54.272	60	54.272	90.45
	24.620	22.5	24.620	109.42
	0.000	--	--	--

3	346.058	360	346.058	96.13
	55.379	60	55.379	92.30
	20.898	22.5	20.898	92.88
	0.000	--	--	--

6. Hook Effect



Not be affected by the concentration of IL-23 up to 5000 pg/mL.

7. Sample Values

Human monocyte-derived macrophages (MDM) were obtained from PBMCs and attached monocytes were cultured in RPMI supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and 100 ng/mL of recombinant human M-CSF for 5 days. Cells were treated with 1 µg/mL LPS and 40 ng/mL of recombinant human IFN-γ overnight.

Condition	Day 1 Mean (pg/mL)	Day 3 Mean (pg/mL)	Day 5 Mean (pg/mL)
Unstimulated	NR	NR	NR
Stimulated with LPS	162.060	43.592	NR

TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts