Human IL-6 ELISA Kit

User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Product No.: 1402431
Version: A/0
For Research Use Only

Huzhou Shenke Biotechnology Co., Ltd

■ Product Name

Human IL-6 ELISA Kit

■ Package

96 tests/Kit

■ Intended Use

This kit is intended for use in determining the presence of natural or recombinant human Interleukin-6 (hIL-6) in biological products, such as serum or cell culture supernatant.

The kit is for RESEARCH USE ONLY and is not intended for clinical use.

■ Product Description

This kit is based on the solid-phase enzyme-linked immunosorbent assay (ELISA) with a double-antibody sandwich technique to determine the amount of hIL-6 in biological products. A hIL-6-specific capture antibody was pre-coated into each well of microtiter strips. Both Calibration Standards and test samples were simultaneously added to the microtiter strips, and followed by incubation and washing. The biotinylated detection antibody was added to the microtiter strips to bind the epitope of hIL-6, thus forming a sandwich structure, which further reacted with streptavidin-labeled HRP (SA-HRP). TMB substrate was added into reaction, catalyzed by enzymatic hydrolysis to produce a blue colored product (maximum absorption peak at 655 nm). Finally, a stop solution was added to terminate the enzymatic reaction, resulting in a yellow colored product (maximum absorption peak at 450 nm). The absorbance values at 450 nm wavelength were positively correlated with the hIL-6 concentration in the Calibration Standards and the samples. The concentration of hIL-6 in the sample can be calculated using a dose-response curve.

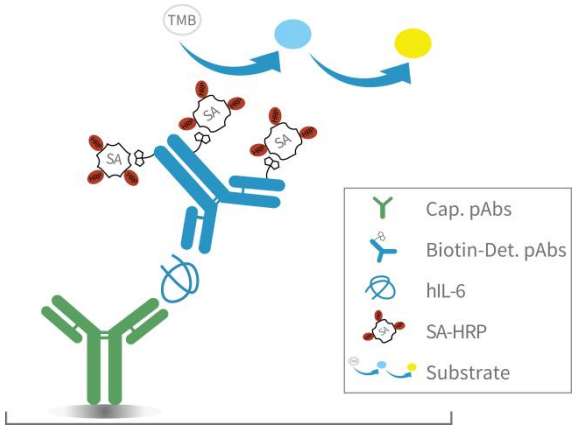


Figure 1. Schematic diagram

■ Kit Contents

Table 1. Kit Components

Reagent	Part No.	Quantity	Note
Anti-hIL-6 Microtiter Strips	PNA019	8 well × 12 strips	Strips pre-coated with mouse anti-hIL-6 monoclonal antibody in a vacuumed bag with desiccant. Seal and store immediately after use.
hIL-6 Calibration Standard	PNB021	1 bottle	Lyophilized powder. Dissolve it with 500 µL Reconstitution Solution, and let it stand for about 5-10 minutes until transparent. Please refer to the details on the label of the tube.
Reconstitution Solution	PNC002	1 × 1.5 mL	Only used for dissolving hIL-6 Calibration Standard.
Diluent	PNE004	2 × 25 mL	For dilution of Calibration Standard, Anti-hIL-6: Biotinylated Conjugate (200×), Streptavidin-HRP (100×) and samples.
Wash Buffer Concentrate (10×)	PNF001	2 × 25 mL	Dilute 10 times with freshly prepared ultra-pure water for plate washing.
Anti-hIL-6: Biotinylated Conjugate (200×)	PNG010	1 × 60 µL	Biotinylated mouse chimeric anti-hIL-6 monoclonal antibody in a protein matrix with preservative. Dilute 200 times with Diluent before use.
Streptavidin-HRP (100×)	PNH002	1 × 140 µL	Streptavidin labeled with HRP. Dilute 100 times with Diluent before use.
TMB Substrate	PND004	1 × 12 mL	Sealed and keep away from light. Equilibrate to room temperature (RT) for 20 minutes before use.
Stop Solution	PNI002	1 × 6 mL	Avoid direct contact with eyes, skin, and clothing.
Sealing Film	PNK001	3 pieces	Cover the strips during incubation to prevent contamination and liquid evaporation.

Note: Room temperature refers to 25 ± 3°C.

■ Storage Conditions

Store the kit at 2-8℃. Please check the expiration date on the labels. The opened components should be stored as shown in Table 2.

Table 2. Recommended storage conditions for opened components

Component	Stability
Anti-hIL-6 Microtiter Strips	Store in the bag with desiccant at 2-8℃ for up to 90 days.
Reconstituted hIL-6 Calibration Standard	Aliquot and store below -18℃, avoid freeze-thaw cycles.

■ Materials Required But Not Provided

- Sterile centrifuge tubes for dilution
- Absorbent paper for plate drying
- Pipette Tips: 1000 μL, 100 μL and 10 μL
- Multi-channel reagent reservoirs (50 mL)

■ Equipment

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 620 nm to 650 nm.
- Single or multi-channel Pipettes: 1000 μL, 100 μL and 10 μL
- Microplate thermoshaker
- Incubator (optional)
- Plate washer (optional)

■ Workflow

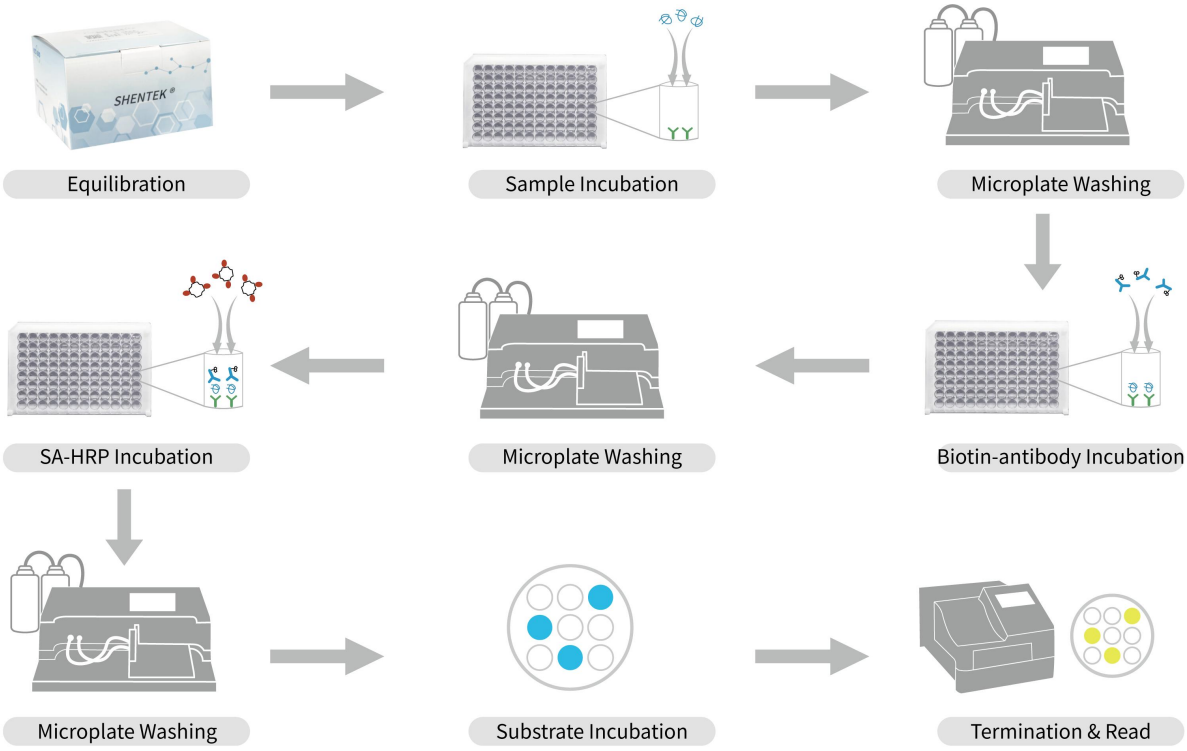


Figure 2. Procedure Flowchart

1. Preparation

(1) Equilibration

- Allow the kit to equilibrate at room temperature for 20 minutes before use. Return to 2-8℃ after use.
- Take appropriate amount of strips to a strip holder according to your experimental design. Please store the remaining strips in the bag with desiccant at 2-8℃.

(2) Preparation of Reagents

- hIL-6 Calibration Standard Solution: Pipette 500 µL of Reconstitution Solution into the bottle containing hIL-6 Calibration Standard. Gently invert 3-5 times to mix well and let it stand for 5-10 minutes. Save the remaining solution under the recommended condition.

  Note: Do not use any other volumes of Reconstitution Solution to dissolve the Calibration Standard.

- 1×Wash Buffer: Dilute 1 volume of Wash Buffer Concentrate (10×) with 9 volumes of ultra-pure water. For example, add 25 mL Wash Buffer Concentrate (10×) to 225 mL of ultra-pure water to make 250 mL of 1×Wash Buffer. Mix well before use.

  Note: If the Wash Buffer Concentrate (10×) or Diluent is cloudy or contains precipitates, heat at 37℃ until it clears.

- 1×Anti-hIL-6: Biotinylated Conjugate: Dilute the Anti-hIL-6:Biotinylated Conjugate (200×) with Diluent in a sterile centrifuge tube to prepare the 1×Anti-hIL-6:Biotinylated Conjugate, mix gently and prepare it freshly.
- 1×Streptavidin-HRP: Dilute the Streptavidin-HRP (100×) with Diluent in a sterile centrifuge tube to prepare the 1×Streptavidin-HRP, mix gently and prepare it freshly.

(3) Preparation of Calibration Standard Solutions

- Prepare hIL-6 Calibration Standard Solutions as shown in Fig 3 and Table 3.

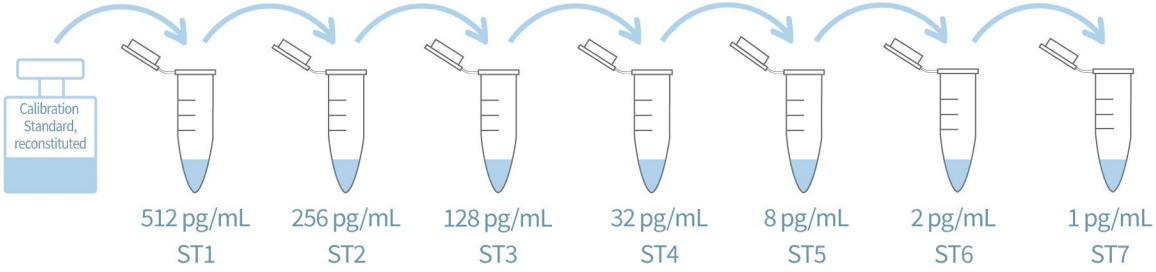


Figure 3. Graphic scheme of hIL-6 Calibration Standard Solutions

Table 3. Preparation of hIL-6 Calibration Standard Solutions

Tubes	Dilution procedure	Conc. (pg/mL)
ST1	Dilute the reconstituted hIL-6 Calibration Standard to ST1 with Diluent	512
ST2	400 µL ST1 + 400 µL Diluent	256
ST3	400 µL ST2 + 400 µL Diluent	128
ST4	200 µL ST3 + 600 µL Diluent	32
ST5	200 µL ST4 + 600 µL Diluent	8
ST6	200 µL ST5 + 600 µL Diluent	2
ST7*	400 µL ST6 + 400 µL Diluent	1
NCS	Diluent	0

*Anchor point

(4) Sample Preparation

- Test samples: In-process samples, harvested bulk, drug substance and drug product. Make sure samples are clear and transparent, and insoluble substances need to be removed by centrifugation or filtration.
- Dilute the samples with a suitable diluent to achieve a proper range of hIL-6 concentration within the calibration curve.
- For the first use, a method validation is recommend to verify sample suitability before the subsequent routine test. This will help to set up appropriate sample dilution series.

Note: Please contact us for support of validation protocol.

2. Assay Experiment

(1) Sample Loading & Incubation

- Pipette 100 µL of Calibration Standard Solutions, NCS (Diluent) and samples into each designated well according to the experimental design. Avoid foaming bubbles during pipetting. We recommend to prepare 2-3 replicates for each concentration.
- Seal the plate and incubate on a microplate thermoshaker at 600 rpm for 1 hour at room temperature, and protect from light.

Table 4. Example of 96-well microplate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	NCS	NCS	NCS									
B	ST7	ST7	ST7									
C	ST6	ST6	ST6	S1	S1	S1						
D	ST5	ST5	ST5	S2	S2	S2						
E	ST4	ST4	ST4	S3	S3	S3						
F	ST3	ST3	ST3	S1+SRC	S1+SRC	S1+SRC						
G	ST2	ST2	ST2	S2+SRC	S2+SRC	S2+SRC						
H	ST1	ST1	ST1	S3+SRC	S3+SRC	S3+SRC						

✧ “ST1-ST7” indicates 7 concentration gradients, “NCS” as the negative control, “S1-S3” as the test samples, and “S1+SRC-S3+SRC” as the spiked recovery controls for each sample.

✧ The number of replicates and the spiked samples can be determined by method validation.

(2) Biotinylated Antibody Incubation

- Wash the plate with 300 μL of 1×Wash Buffer per well. Wipe off any liquid from the bottom outside of the plate. Repeat the washing for 3 times. Do not allow the wells to be completely dry before adding the next solution.
- Pipette 100 μL of 1×Anti-hIL-6:Biotinylated Conjugate into the corresponding wells as indicated earlier.
- Seal the plate and incubate at room temperature for 45 minutes, and protect from light.

(3) Streptavidin-HRP Incubation

- Wash the plate with 300 μL of 1×Wash Buffer per well. Wipe off any liquid from the bottom outside of the plate. Repeat the washing for 3 times. Do not allow the wells to be completely dry before adding the next solution.
- Pipette 100 μL of 1×Streptavidin-HRP into the corresponding wells.
- Seal the plate and incubate at room temperature for 30 minutes, and protect from light.

(4) TMB Reaction

- Equilibrate the TMB Substrate at room temperature for 20 minutes.
- Wash the plate with 300 μL of 1×Wash Buffer per well. Wipe off any liquid from the bottom outside of the plate. Repeat the washing for 5 times. Do not allow the wells to be completely dry before adding the substrate.
- Add 100 μL of TMB Substrate into the wells, and incubate at room temperature for 10 minutes, and protect from light.

  Note: Do not use sealing film during this step.

(5) Termination & Reading

- Add 50 μL of Stop Solution into each well.

  Note: The order of adding Stop Solution should be the same as the order of adding the TMB Substrate. While adding samples, suspend the tips above the liquid to prevent contact with the solution in the wells and minimize the risk of bubble formation.

- Read absorbance at 450 nm/620-650 nm. Plate reading should be completed within 10 minutes after termination.

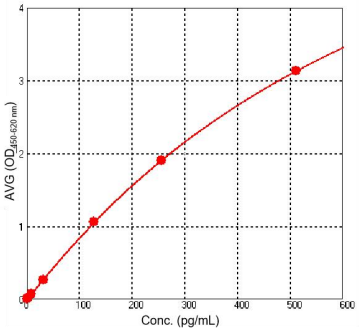
3. Calculation and Analysis

- The OD_{450nm} value of each well should be calculated by subtracting their respective long wavelength, as of $OD_{620\ nm}$ in this case. If the microplate reader is not equipped with long wavelength measurement, this step can be omitted.
- The $OD_{450\text{-}620\text{-}N}$ value of calibration curve fitting points and samples should be calcualted by substracting the $OD_{450\text{-}620}$ of NCS, then take the average value of replicates.
- Perform a 4-parameter logistic regression model using the Calibration Standard concentration values and OD values to obtain the calibration curve equation. The data analysis software for calibration curve fitting could be the built-in software from microplate reader. If not, we recommend to use professional standard curve software such as Curve Expert, ELISA Calc, and so on.
- Substitute the average OD value of the samples into the equation to calculate the sample concentration.
- For samples with absorbance values above the Calibration Standard ST1, a pilot study should be conducted to determine an appropriate dilution. The HCP concentration in the samples are calculated from the test value multiplied by its corresponding dilution factor. If the spiked samples are simultaneously set at this dilution level, and the recovery rate should meet the requirements of the corresponding regulations.

■ Assay Performance

- Linearity & Range: 2-512 pg/mL, $R^2 \geq 0.990$
- LLOQ: 2 pg/mL
- Specificity: No cross-reactivity with IL-2, IL-10, IL-12, IFN-γ, IFN-β, IL-6Rα, Rat IL-6 and Mouse IL-6.
- Typical calibration curve for reference:

Calibration Standards (ng/mL)	Abs. At (450 nm-620 nm)	AVG
512	3.238	3.158
	3.097	
	3.138	
256	1.916	1.929
	1.954	
	1.918	
128	1.072	1.087
	1.172	
	1.018	
32	0.289	0.294
	0.295	
	0.298	
8	0.092	0.095
	0.094	
	0.099	
2	0.042	0.041
	0.040	
	0.040	
1	0.032	0.032
	0.032	
	0.033	
0	0.024	0.024
	0.024	
	0.024	



4-PL: $Y = \frac{A-D}{1+(\frac{X}{C})^B} + D$

A = 7.86930
B = -1.04651
C = 760.10625
D = 0.00086
R^2 = 0.99999

■ Additional Information

- ✧ This kit is intended for use by qualified technicians only.
- ✧ Consumables, for example sterile disposable tips, tubes and reservoirs are only allowed for single use. It is recommended to wipe with 75% ethanol before and after each use. Follow the specified pipetting procedure carefully.
- ✧ Users should validate the assay before testing their samples.
- ✧ Dilution should be gentle and thorough to avoid excessive foaming.
- ✧ Stop Solution is 1M HCl. Avoid direct contact with eyes, skin, and clothing.
- ✧ Do not mix the kit reagents from different lot numbers.
- ✧ Use fresh sterile water or ultra-pure water, and ensure the water temperature does not exceed 37℃.
- ✧ Seal or cover the microplate immediately after sample loading to avoid liquid evaporation.
- ✧ Avoid drying the wells before substrate incubation.
- ✧ Store unused microtiter strips in a sealed bag with desiccant to prevent contamination.
- ✧ Centrifuge Anti-hIL-6:Biotinylated Conjugate (200×) and Streptavidin-HRP (100×) before use to avoid any loss of the reagent.
- ✧ To avoid pipetting errors, pipetting or sampling accurately for dilution of Standard and samples, for example, a minimum volume of 5 μL is recommended.
- ✧ hIL-6 Calibration Standard Solutions, 1×Anti-hIL-6:Biotinylated Conjugate and 1× Streptavidin-HRP are recommended for single use due to stability issue. Prepare freshly before each experiment.
- ✧ TMB Substrate should be colorless. If not, discard it and contact us for assistance.
- ✧ Pipette carefully to avoid any bubbles, and gently shake the plate for thorough mixing. Bubbles can influence optical density values and detection results.
- ✧ Avoid the samples containing sodium azide (NaN_3), which will deactivate the HRP and lead to the underestimation of HCP levels.

■ Troubleshooting

Problem	Possible Cause	Solution
High background signal (OD)	Cross-contamination of reagents, including distilled water	Freshly prepared prior to experiment.
	Cross-contamination of equipment, including micropipette and centrifuges	Clean the equipment with 75% ethanol before experiment.
	Environment contamination	Separate the working bench to avoid contamination.
	Insufficient washing	Increase the wash buffer volume or wash times, and remove any remaining liquid before proceeding to the next step.
Abnormal values	Improper washing	Swiftly and completely shake off any excess liquid, and avoid reusing paper towels to minimize contamination.
	Improper sampling	Add the samples to the bottom of the wells using micropipettes, and avoid splashing to the neighboring wells.
	Plate sealing	Promptly cover the plate with the sealing film and remove it carefully to prevent splashing.

If you have any other questions, please contact us for technical support.

■ References

- ICH. M10 Bioanalytical Method Validation And Study Sample Analysis
- FDA. Bioanalytical Method Validation Guidance for Industry
- ChP<9012>Guidance of Quantitative Method Validation for Biological Samples

Effective date: 04 Apr. 2025

Support & Contact

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