Human IL-1β ELISA Kit

Please read the manual carefully before use.

Cat. No.: NE108

Storage: at 2~8°C in the dark for one year.

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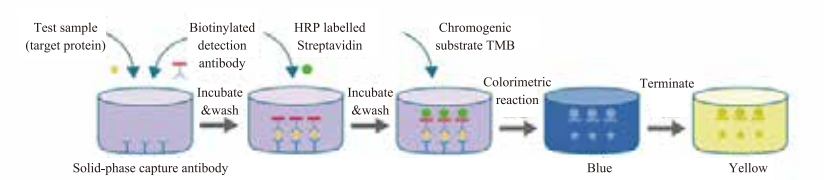
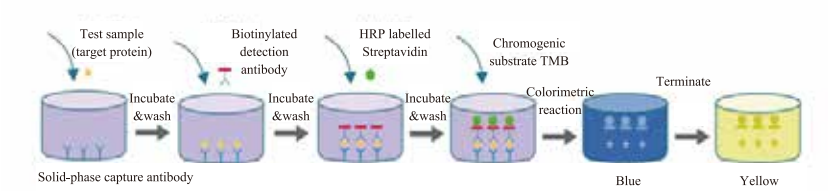
Description

Interleukin-1β (IL-1β) is a pro-inflammatory cytokine primarily secreted by activated macrophages, monocytes, and the slanDC dendritic cell subset in the form of a protein precursor. It becomes an active form through hydrolysis by caspase 1 (CASP1/ICE). IL-1β plays a regulatory role in cell immune activation and the inflammatory response. It stimulates CD4+ cells to differentiate into Th17 cells. When combined with IL-23, IL-1β induces γδ T cells to express IL-17, IL-21, and IL-22. Together with IL-1α and IL-18, IL-1β coordinates immune responses through various downstream mechanisms. IL-1β is involved in the regulation of IL-6 and TNF-α, activating the adhesion molecule ICAM1. IL-1β participates in the body's hematopoietic system, nervous system, endocrine system, inflammatory responses, and certain anti-tumour physiological processes, including sepsis, acute and chronic myeloid leukaemia, atherosclerosis, type II diabetes, rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, multiple sclerosis, Crohn's disease, Alzheimer's disease, melanoma, colon cancer, lung cancer, breast cancer, etc.

This assay kit employs enzyme-linked immunosorbent assay (ELISA) method with dual antibodies to quantitatively determine the concentration of IL-1β in human serum, plasma, and cell culture supernatant. The kit utilizes a high-affinity anti-human IL-1β antibody pre-coated on the enzyme-labelled plate. Standard samples or test samples are added to the microplate wells, and after incubation, IL-1β present in the samples specifically binds to the pre-coated antibody on the plate. After washing to remove unbound substances, biotin-labelled anti-human IL-1β detection antibody is added to the microplate wells. Following another incubation, the detection antibody binds specifically to IL-1β anchored on the enzyme-labelled plate. Subsequently, streptavidin-horseradish peroxidase (Streptavidin-HRP) is added to the microplate wells and incubated. The high-intensity non-covalent binding between biotin on the antibody and streptavidin forms a "pre-coated antibody - human IL-1β protein - detection antibody - Streptavidin-HRP" immunocomplex. After another wash, the chromogenic substrate TMB is added to the microplate wells, and HRP catalyses the generation of a blue product. The intensity of the chromogenic reaction is directly proportional to the concentration of IL-1β in the sample. The reaction is terminated by adding a stop solution, and the absorbance is measured at 450 nm wavelength (reference wavelength 570 - 630 nm). By plotting a standard curve, the concentration of IL-1β in the sample can be calculated from the absorbance values. This assay kit is highly specific, sensitive, and convenient to operate.



Cell Culture Supernatant - Sandwich ELISA Principal Diagram with Dual Antibodies



Serum, Plasma - Sandwich ELISA Principal Diagram with Dual Antibodies

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Suitable Sample Types

Cell culture supernatant, serum, plasma, etc.

Kit Contents

Component	NE108-01	Storage
Human IL-1β Antibody Precoated ELISA Plate	96T	at 2~8˚C
Human IL-1β Standard	2 bottles	at 2~8˚C
Standard & Sample Diluent	15ml/bottle	at 2~8˚C
100× Human IL-1β Detection Antibody	120μl/test	at 2~8˚C
100× Streptavidin-HRP	120ul/test	at 2~8˚C in the dark
Detection Antibody & Streptavidin-HRP Diluent	25ml/bottle	at 2~8˚C
20× Washing Buffer	50ml/bottle	at 2~8˚C
TMB Chromogenic Substrate	12ml/bottle	at 2~8˚C in the dark
Stop Solution	6ml/bottle	at 2~8˚C
Plate Sealers	4 pieces	

Note: The used kit can be stored at 2~8˚C for 1 month; please use the unused kit within 1 year.

Self-prepared regents and equipment

1.Deionized water.
2.EP tubes, pipettes, tips, graduated cylinder, etc.
3.Microplate shaker.
4.Automatic plate washer or 8-channel manual plate washer or multi-channel pipette.
5.ELISA reader: 450nm main wavelength, 620nm reference wavelength.

Sample collection

1.Cell culture supernatant

Centrifuge the cell culture at 300×g, 4˚C for 10 minutes and collect the supernatant. Aliquot the supernatant into EP tubes in equal amounts and store at -20˚C. Avoid repetitive freeze-thaw cycles (samples can be stored at 2~8°C for testing within 24 hours).

2.Serum samples

Allow blood sample to naturally clot at room temperature for 30 minutes. Centrifuge at 1000×g, 4˚C for 10 minutes, then aliquot the supernatant into EP tubes in equal amounts and store at -20˚C. Avoid repetitive freeze-thaw cycles (samples can be stored at 2~8˚C for testing within 24 hours).

3.Plasma samples

For anticoagulation, EDTA or sodium citrate is recommended. Collect whole blood into anticoagulant-containing collection tubes. Allow it to stand at room temperature for at least 20 minutes. Centrifuge at 1000×g, 4˚C for 10 minutes. Aliquot the supernatant into EP tubes in equal amounts and store at -20˚C. Avoiding repetitive freeze-thaw cycles (samples can be stored at 2~8˚C for testing within 24 hours).

Note: Haemolyzed and hyperlipidaemic serum or plasma samples may affect the accuracy of test results and should be avoided if possible.

Sample dilution

1. For serum/plasma samples, it is recommended to dilute them 2-fold with the Standard & Sample Diluent before testing, for example: 100 μl serum + 100 μl Standard & Sample Diluent.
2. If the positive value of the cell culture supernatant sample falls within the curve range, no dilution is needed, and it

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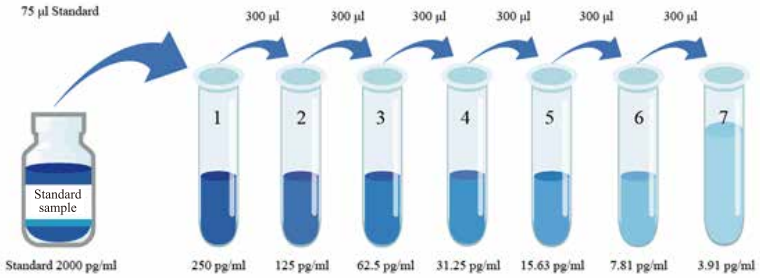
can be tested directly. If the positive value exceeds the curve range, it is necessary to dilute with the Standard & Sample Diluent to bring it within the curve range for testing. When calculating the concentration, it should be multiplied by the corresponding dilution factor.

3. It is advisable to conduct a pre-experiment to determine the sample dilution factor before the formal experiment.

Working solution preparation

Before preparation, please bring all reagents to room temperature.

1. 1× Human IL-1β Detection Antibody: Prior to use, briefly centrifuge to ensure liquid consolidation at the bottom of the tube. Dilute 100× human IL-1β detection antibody with detection antibody & streptavidin-HRP dilution buffer to a 1× working concentration, based on the quantity needed for the experiment. Use within 15 minutes of preparation.
2. 1× Streptavidin-HRP: Prior to use, briefly centrifuge to ensure liquid consolidation at the bottom of the tube. Dilute 100× streptavidin-HRP with detection antibody & streptavidin-HRP dilution buffer to a 1× working concentration, based on the quantity needed for the experiment. Use within 15 minutes of preparation.
3. 1× Wash Buffer: Dilute 20× Wash Buffer with deionized water to a 1× working concentration based on the required amount for the experiment. Store at 2~8°C for up to 30 days after preparation.
4. Human IL-1β standard preparation: Dissolve the lyophilized standard according to the specified volume on the standard label, using the corresponding volume of Standard & Sample Diluent. The concentration of the dissolved standard is 2000 pg/ml. Use the dissolved standard within 30 minutes.
5. Standard curve gradient dilution: Dilute the dissolved standard 8-fold with Standard & Sample Diluent. Take 75 µl of the dissolved standard and add it to 525 µl of Standard & Sample Diluent; mix well, designated as tube 1 (concentration: 250 pg/ml). Perform a 2-fold gradient dilution in Tubes 2-7 as illustrated in the diagram. Tube 2 concentration: 125 pg/ml, Tube 3 concentration: 62.5 pg/ml, and continue the gradient dilution to Tube 7 (concentration: 3.91 pg/ml). The highest point (250 pg/ml) serves as the upper limit of the standard curve, and the Standard & Sample Diluent represents the zero point (0 pg/ml), i.e., the blank value.



Experimental Protocol

1. Before the assay, ensure all reagents are brought to room temperature. Retrieve the required strips for the current experiment, and promptly seal any unused strips in an aluminium foil bag for storage at 2~8°C.
2. Add the diluted standards and samples to their respective wells, 100 µl per well.
   For cell culture supernatant samples: Add 1× Detection Antibody to all wells, 100 µl per well. Shake on a microplate shaker for 30 seconds, cover with sealing membrane, and incubate at room temperature for 2 hours.
   For serum and plasma samples: Shake on a microplate shaker for 30 seconds, cover with sealing membrane, and incubate at room temperature for 2 hours. It is recommended to perform duplicate tests for standards and samples, maintaining consistent order of adding reagents for consistent results.

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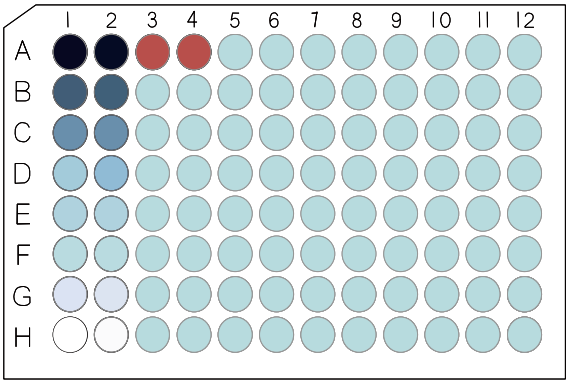
3. Discard the liquid in the wells, wash the plate with 1× wash buffer, 300 µl per well. It is advisable to shake on a microplate shaker for 30 seconds before discarding the wash solution. Repeat this process five times, with the final step involving patting the plate dry on absorbent paper.
   For cell culture supernatant samples: Skip steps 4 and 5, proceed to step 6.
   For serum and plasma samples: Proceed to step 4.
4. Add 1× detection antibody to all wells, 100 µl per well. Shake on a microplate shaker for 30 seconds, cover with sealing membrane, and incubate at room temperature for 1 hour.
5. Repeat step 3.
6. Add 1× Streptavidin-HRP to all wells, 100 µl per well. Shake on a microplate shaker for 30 seconds, cover with sealing membrane, and incubate at room temperature for 30 minutes.
7. Repeat step 3.
8. Add TMB substrate to all wells, 100 µl per well. Shake on a microplate shaker for 30 seconds, cover with sealing membrane, and incubate at room temperature for 20 minutes.
9. After incubation, add stop solution, 50 µl per well. Read the absorbance at a wavelength of 450 nm, with a reference wavelength of 620 nm.
10. After completing the experiment, return any unused reagents and the ELISA plate frame to the kit and store at 2~8°C. Use up the kit within one month is recommended.

ELISA plate sample loading



Note: A1/A2: 100µl 250pg/ml standard
B1/B2: 100µl 125pg/ml standard
C1/C2: 100µl 62.5pg/ml standard
D1/D2: 100µl 31.25pg/ml standard
E1/E2: 100µl 15.63pg/ml standard
F1/F2: 100µl 7.81pg/ml standard
G1/G2: 100µl 3.91pg/ml standard
H1/H2: 100µl 0pg/ml standard (standards & sample diluents)
A3/A4: 100µl sample

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Result analysis

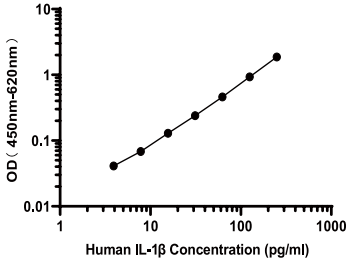
1. Perform dual-wavelength detection using a microplate reader to measure the optical density (OD) values at the main wavelength of 450 nm and the reference wavelength of 620 nm. The OD value is obtained by subtracting the OD measurement at 620 nm from the OD measurement at 450 nm.
2. Calculate the average OD value for duplicate wells of the standard, then subtract the blank value (average OD value of the 0 pg/ml standard) to obtain the standard's calibrated value. Use the concentration of the standard as the x-axis and the calibrated OD value as the y-axis to generate a standard curve using linear regression or the four-parameter method.
3. Calculate the concentration of the samples using the sample OD values and the standard curve equation. If the sample OD value exceeds the upper limit of the standard curve, it should be appropriately diluted and retested. When calculating the concentration, multiply by the corresponding dilution factor.

Reference data

Each assay requires the establishment of a standard curve. The following data is provided as reference data for constructing the standard curve.

Cell culture supernatant standard curve

Standard pg/ml	OD value		Average	Calibrated value
250	1.814	1.915	1.8645	1.851
125	0.934	0.93	0.9320	0.918
62.5	0.479	0.444	0.4615	0.448
31.25	0.247	0.23	0.2385	0.225
15.63	0.126	0.132	0.1290	0.115
7.81	0.065	0.071	0.0680	0.054
3.91	0.042	0.04	0.0410	0.027
0	0.015	0.013	0.0140	0.000

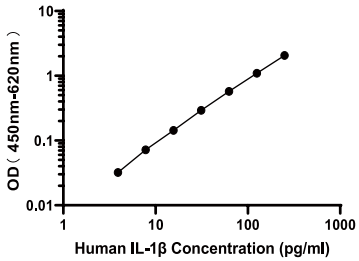


Interpretation of standard curve results for the duplicate wells:

In the table above, the average OD value for the duplicate wells of the 0 pg/ml standard is calculated as (0.015+0.013)/2 = 0.014, and the calibrated value is set to 0. For the 250 pg/ml standard, the average OD value for the duplicate wells is calculated as (1.814+1.915)/2 = 1.8645. Therefore, the calibrated value for the 250 pg/ml standard is 1.8645 - 0.014 = 1.851.

Serum and plasma standard curve

Standard pg/ml	OD value		Average	Calibrated value
250	2.048	2.095	2.0715	2.056
125	1.096	1.127	1.1115	1.096
62.5	0.581	0.598	0.5895	0.574
31.25	0.304	0.312	0.3080	0.293
15.63	0.156	0.16	0.1580	0.143
7.81	0.086	0.089	0.0875	0.072
3.91	0.046	0.049	0.0475	0.032
0	0.016	0.015	0.0155	0.000



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Interpretation of standard curve results for the duplicate wells:
In the table, the average OD value for the duplicate wells of the 0 pg/ml standard is calculated as (0.016+0.015)/2 = 0.0155, and the calibrated value is set to 0. For the 250 pg/ml standard, the average OD value for the duplicate wells is calculated as (2.048+2.095)/2 = 2.0715. Therefore, the calibrated value is 2.0715 - 0.0155 = 2.056.

Precision

Intra-plate Precision:

Measure 20 replicate wells on a single ELISA plate for three replicates with known concentrations, to assess the precision within the same plate.

Inter-plate Precision:

Measure 20 replicate wells on different ELISA plates for three replicates with known concentrations, to evaluate the precision between different plates.

	Intra-plate			Inter-plate		
	1	2	3	1	2	3
Average (pg/ml)	105.1	53.7	24.2	106.3	52.4	23.8
Standard Deviation	4.5	2.9	1.2	4.9	2.5	1.7
Coefficient of Variation (%)	4.2	5.4	4.9	4.7	4.8	7.3

Recovery rate

Different concentration of IL-1β were added to the serum, plasma and cell supernatant of 4 healthy people, and the samples without IL-1β were used as the background to calculate the recovery rate.

Sample type	Average Recovery (%)	Range (%)
Serum	101	89~114
EDTA Plasma	98	85~116
Sodium Citrate Plasma	95	91~118
Cell Supernatant	103	95~113

Linear recovery rate

High concentrations of human IL-1β were added to the serum and plasma of 4 healthy people, and linear dilution was performed to detect the linear recovery rate.

Dilution Rate	Recovery Rate	Serum	EDTA Plasma	Sodium Citrate plasma	Cell Culture Supernatant
1:2	Average (%)	98	96	90	100
	Range (%)	95~105	87~109	83~107	92~106
1:4	Average (%)	101	98	95	102
	Range (%)	92~107	90~112	85~110	90~109
1:8	Average (%)	100	101	99	101
	Range (%)	89~111	85~114	91~113	93~112
1:16	Average (%)	102	99	100	103
	Range (%)	90~113	86~117	84~116	91~115

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Calibration

The standard sample of this kit is high-purity recombinant human IL-1β calibrated by TransGen Biotech.

Sensitivity

The minimum detectable concentration of human IL-1β is 2.0 pg/ml. Sensitivity is the corresponding concentration calculated from the mean of 20 replicate zero standard OD values plus two standard deviations.

Sample value

Using this reagent kit, the levels of IL-1β were detected in 30 serum samples from healthy individuals, and the detected values for all 30 samples were below 2.0 pg/ml.

Specificity

The reagent kit specifically recognizes natural and recombinant human IL-1β. Evaluation using recombinant human cytokines such as EGF, FGF-basic, GM-CSF, IFN-γ, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 p70, IL-12B, IL-23, TNF-α has been conducted to assess specificity. No cross-reactions or interference effects were observed with these cytokines.

Notes

1. Store this reagent kit in dark place at 2~8°C. Do not use the kit one month after opening.
2. To ensure accurate results, a standard curve should be prepared for each test.
3. Thoroughly mix all the reagents used in the experiment.
4. After each plate-washing, pat dry on absorbent paper. If there are bubbles in the wells, they can be pierced by a pipette tip. Use a new tip for each well to avoid cross-contamination.
5. The TMB colour development substrate is a colourless and transparent liquid. Do not use if there is a colour change.
6. After TMB colour development, judge whether to add the stop solution earlier or later based on the intensity of the colour.
7. After adding the stop solution, complete the reading within 30 minutes.
8. It is recommended to read at the main wavelength of 450 nm with a reference wavelength of 620 nm. If only a single wavelength of 450 nm is used for reading, the OD values may be generally higher, and the blank values may increase, leading to a decrease in the accuracy of the reagent kit.
9. The stop solution in the kit is corrosive. Operators should wear gloves and take protective measures. In case of contact, rinse with plenty of water and seek medical attention promptly.
10. To avoid cross-contamination, use a new tip for each test sample and standard sample. Use disposable test tubes, pipette tips, plate seals, and clean plastic containers for the experiment.
11. Components from different batches or sources of the reagent kit should not be mixed.

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