Mouse IL-1β ELISA Kit

Product Description

Mouse interleukin 1β (Mouse IL-1β/IL-1F2), as a major member of the IL-1 family, is primarily produced by activated macrophages. It stimulates thymocyte proliferation through induction of IL-2 release, promotes B-cell maturation and proliferation, and activates fibroblast growth factor to stimulate thymocyte proliferation. It can also stimulate synovial cells to release prostaglandins and various collagenases. IL-1β plays a central role in physiological phenomena such as immunity and inflammation, bone remodeling, fever, and carbohydrate metabolism. Aberrant IL-1β signaling drives tumorigenesis by promoting epithelial-to-mesenchymal transition, increasing production of inflammatory cytokines and chemokines, inducing immune suppression and resistance to cell apoptosis, and increasing leukocyte adhesion. IL-1β-induced inflammation-related diseases include diabetes, arthritis, and atherosclerosis. Moreover, abnormal activation of IL-1β is also associated with poor prognosis in most cancer types, including colon cancer, lung cancer, and breast cancer, among others.

The Arcegen Mouse IL-1β/IL-1F2 ELISA (Enzyme-Linked Immunosorbent Assay) Kit is an in vitro enzyme-linked immunosorbent assay kit used for quantitative determination of Mouse interleukin 1β (Mouse IL-1β/IL-1F2) in serum and plasma. Specific antibodies against Mouse interleukin 1β are pre-coated on a high-affinity ELISA plate. Standard samples and test samples are added to the wells of the ELISA plate, and after incubation, Mouse interleukin 1β present in the samples binds to the solid-phase antibody. After washing to remove unbound substances, a detection antibody is added for incubation and binding, followed by washing and addition of enzyme conjugate (Streptavidin-HRP) for incubation and binding. After washing, a colorimetric substrate TMB is added for color development under light-shielding conditions. The intensity of the color reaction is directly proportional to the concentration of Mouse interleukin 1β in the samples. The reaction is terminated by adding stop solution, and the absorbance is measured at 450 nm wavelength (reference wavelength 570~630 nm).

Specifications

Item Number	P162004S / P162004E
Specification	48 T / 96 T
Detection Range	15.63~1000 pg/mL
Detection Method	Sandwich ELISA
Detection Time	4.5 hours
Sensitivity	11.46 pg/mL
Dilution Linearity	89~123%

Recovery Rate	78~107%
Intra-assay Variability	4.0%
Inter-assay Variability	3.9%

Components

Component Number	Component Name	Storage Temperature	P162004S	P162004E
P162004-A	ELISA Plate	2~8°C	48 T	96 T
P162004-B	Standard	2~8°C	1 tube	2 tubes
P162004-C	Detection Antibody	2~8°C	120 µL	240 µL
P162004-D	Enzyme Conjugate	2~8°C(Avoid Light)	30 µL	60 µL
P162004-E	Sample Dilution Buffer	2~8°C	8 mL	15 mL
P162004-F	Antibody/Enzyme Dilution Buffer	2~8°C	15 mL	30 mL
P162004-G	20x Wash Buffer	2~8°C	25 mL	50 mL
P162004-H	Substrate Solution	2~8°C(Avoid Light)	8 mL	15 mL
P162004-I	Stop Solution	Room Temperature	5 mL	10 mL
P162004-J	Plate Sealant Film	Room Temperature	3 pieces	5 pieces

Shipping and Storage

Reagent Kit can be stored at 2~8°C or according to the storage conditions provided for each component to prevent contamination and repeated freeze-thaw cycles. Dilute reagents to working concentrations immediately before use and discard them afterward; they should not be reused. The shelf life is 1 year.

Table 1 Reagent Storage Table After Initial Use

Material Name	Storage Conditions
Enzyme-linked immunosorbent assay (ELISA)	Unused strips can be returned to the aluminum foil bag, tightly sealed, and stored at 2~8°C to avoid moisture absorption.
plate Standard sample	Use within 48 hours after dissolution, store at 2~8°C to avoid contamination.

Detecting antibody	Use within 48 hours after dilution, store at 2~8°C to avoid contamination.
Enzyme conjugate	
Sample diluent	Store at 2~8°C for 1 month, avoiding contamination.
20×Wash solution	
Antibody/enzyme diluent	Store at 2~8°C for 1 month, avoiding light exposure.
Substrate solution	
Stop solution	Can be stored at room temperature.
Plate seal film	

Operating precautions

1. For your safety and health, please wear laboratory attire and disposable gloves while handling.
2. The reagent kit should be used within its expiration date. Mixing related reagents from different batches is prohibited.
3. This product is only intended for the detection of target antigens as indicated in the instruction manual with specific samples. Other applications require validation by the user, and the reliability and accuracy should be evaluated based on the results.
4. Do not mix or substitute reagents or materials from different batches of other kits.
5. For research use only.

Instructions for use

1. Used for quantitative detection of IL-1β/IL-1F2 content in serum, plasma, and cell culture supernatants.
2. Please read the instructions carefully before using this product.

Common Technical Tips

1. If the sample OD value is higher than the S1 OD value, further dilution should be performed in an appropriate sample diluent.
2. Avoid foaming during mixing.
3. When adding standards, samples, and others, promptly change pipette tips to prevent cross-contamination.
4. Ensure proper sealing of the microplate or complete covering with sealing film during incubation.
5. Completely remove all solutions and buffers during the washing steps.

6. Do not invert the standard vial before dissolving the standard. After inverting the standard vial, thoroughly mix it by gently tapping or inverting and then centrifuge at low speed after adding the buffer.
7. Follow the instructions for placing reagents during the experiment.
8. Dispose of buffers promptly after use; use them once and discard.
9. Reagent kit components vary among different products and must not be interchanged.

Other Preparation Materials

1. Microplate reader, measuring absorbance at 450 nm (reference wavelength 630 nm).
2. Incubator, automated microplate washer.
3. Pipettors, pipettes ranging from 1 μL to 1 mL with corresponding tips.
4. Graduated cylinders, 100 mL and 1 L.
5. Tubes for standard or sample dilution.
6. Blotting paper.
7. Distilled water or deionized water.
8. Computer and analysis software.

Experiment Preparation

1. Sample Collection and Processing

1) Cell culture supernatant: Centrifuge at 1,000×g for 10 minutes to remove debris, then proceed with immediate detection or aliquot and store at temperatures below -20°C.

2) Serum samples: Collect serum using tubes free from pyrogens and endotoxins. After clotting for 30 minutes, centrifuge at 1,000×g for 10 minutes. Perform immediate detection after aspiration of serum samples or aliquot and store at temperatures below -20°C.

3) Plasma samples: Collect plasma samples using EDTA, sodium citrate, or heparin as anticoagulants. Centrifuge at 1,000×g for 30 minutes to collect samples. Proceed with immediate detection or aliquot and store at temperatures below -20°C.

4) This assay kit may be applicable to other biological samples. Serum, plasma, and cell culture supernatants have been validated.

【Note】Visible precipitates in samples must be removed before testing. Do not use samples with severe hemolysis or high lipid content. Samples should be aliquoted and stored at -20°C to prevent loss of IL-1β/IL-1F2 activity. If testing within 24 hours, samples can be stored at 2~8°C to avoid repeated freeze-thaw cycles. Before testing, frozen samples should be slowly equilibrated to room temperature (25°C±3°C) and gently mixed.

If samples require dilution, use the specified sample diluent for dilution.

Recommended dilutions for normal serum/plasma samples (for reference only): 1:1 dilution with

sample diluent Recommended dilution for cell culture supernatant (for reference only): undiluted Due to variations in target protein content in samples, the dilution ratio for each sample is recommended based on preliminary experiment results or determined based on actual conditions.

2. ELISA Plate Preparation

The ELISA plate should be equilibrated to room temperature before use. Unused strips should be promptly sealed with desiccants and stored at 2~8°C. It is recommended to conduct each sample in duplicate wells.

3. Reagent Preparation

Before use, all reagent components and the test samples need to equilibrate to room temperature. To ensure the accuracy of the experiment, please complete within 15 minutes before use.

1) 1×Wash Buffer Preparation: Equilibrate the concentrated buffer to room temperature until fully dissolved without any crystallization. Mix well, take 25 mL of 20×Wash Buffer and dilute it with distilled water to a total volume of 500 mL. Prepare the specific volume according to the amount needed for each use.

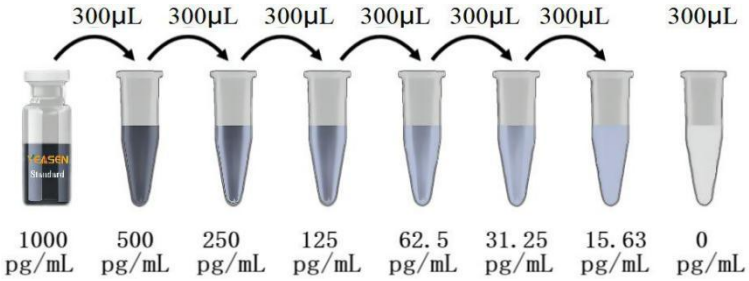
2) Detection Antibody Preparation: Before use, centrifuge at 10, 000 rpm for 20 seconds. Dilute the antibody with Antibody Dilution Buffer at a ratio of 1:50 to the working concentration. For example, take 120 μL and dilute it to a total volume of 6 mL with Antibody Dilution Buffer. Prepare the specific volume according to the amount needed for each use and mix thoroughly.

3) Enzyme Conjugate Preparation: Before use, centrifuge at 10,000 rpm for 20 seconds. Dilute the enzyme with Enzyme Dilution Buffer at a ratio of 1:200 to the working concentration. For example, take 30 μL and dilute it to a total volume of 6 mL with Antibody/Enzyme Dilution Buffer. Prepare the specific volume according to the amount needed for each use and mix thoroughly.

4) Preparation of Standard Curve: Prepare 7 sterile 1.5 mL centrifuge tubes and label them according to the standard concentrations. S1 Preparation: Dissolve one vial of standard lyophilized product in Sample Dilution Buffer according to the labeled amount, mix well to obtain a concentration of 1000 pg/mL. Add 300 μL of 1×Sample Dilution Buffer to each tube, take 300 μL of S1 and mix thoroughly in the first tube, then transfer 300 μL to the next tube for the next dilution, and so on to prepare a 2-fold serial dilution standard curve. The starting highest concentration should be labeled as 1000 pg/mL, and the lowest concentration as 15.63 pg/mL. Prepare the standard curve for each assay, and do not mix standard curves from different kits or different times. When testing samples, add 100 μL of standard to each well. Prepare a higher volume than needed to avoid insufficient volume.

Table 2 Preparation of IL-1β/IL-1F2 Standard Solutions (15.63 ~ 1000 pg/mL)

Standard Curve	Diluent (μL)	Volume of Standard Added (μL)	Final Concentration of Standard (ng/mL)
S1	As labeled	/	1000
S2	300	300	500
S3	300	300	250
S4	300	300	125
S5	300	300	62.5
S6	300	300	31.25
S7	300	300	15.63
Blank	300	0	0



Operating Instructions

Before use, all reagents and test samples need to equilibrate to room temperature. It is strongly recommended to perform duplicate well testing for all standard solutions and test samples.

1. Reagent Preparation: Prepare all necessary reagents, diluted standard solutions, and test samples.
2. Plate Determination: Calculate the number of microplate wells required for test samples and standard solutions. Remove the required microplate wells from the aluminum foil pouch, return the remaining wells to the pouch, and seal the pouch tightly for storage at low temperature.
3. Plate Soaking: Add 1×Wash Buffer (350 μL/well) to soak the microplate wells. After 30 seconds, discard the liquid from the wells and pat dry the microplate. The volume of liquid in the wells significantly affects the assay results, ensure there is no residual wash buffer after the final pat dry.
4. Incubation with Samples: Add various concentrations of standard solutions and diluted test samples to the microplate wells, 100 μL/well. Ensure sample addition is completed within 15 minutes. Incubate at room temperature for 2 hours.
5. Plate Washing: Discard the liquid from the wells and wash the microplate 5 times with 1× Wash Buffer (350 μL/well). Pat dry the microplate.
6. Antibody Incubation: Add detection antibodies prepared to working concentration to the

microplate wells, 100 µL/well. Incubate at room temperature for 2 hours.

7. Plate Washing: Discard the liquid from the wells and wash the microplate 5 times with 1× Wash Buffer (350 µL/well). Pat dry the microplate.
8. Enzyme Conjugate Incubation: Add enzyme conjugate prepared to working concentration to the microplate wells, 100 µL/well. Incubate at room temperature for 20 minutes.
9. Plate Washing: Discard the liquid from the wells and wash the microplate 5 times with 1× Wash Buffer (350 µL/well). Pat dry the microplate.
10. Color Development: Equilibrate the substrate solution to room temperature for 10 minutes before use. Add substrate solution to the microplate wells, 100 µL/well. Incubate at room temperature in the dark for 15 minutes.
11. Termination: Add 50 µL/well of stop solution to the microplate wells. The color will change from blue to yellow. Gently shake the microplate to ensure uniform color development.
12. Reading: Read the absorbance values at 450 nm/630 nm within 10 minutes.

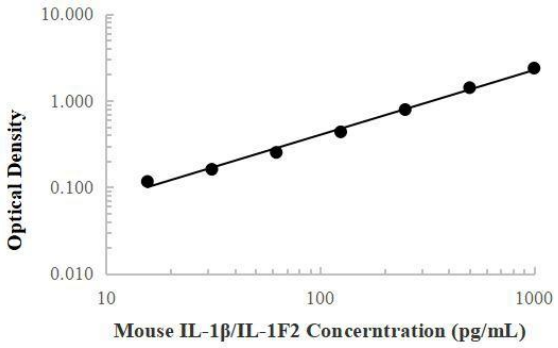
Standard Curve Establishment

Calculate the average OD values of duplicate wells for both standard solutions and samples, then subtract the average OD value of the blank wells to obtain the calibrated OD value. Plot the standard curve with the logarithm of the standard concentrations on the x-axis and the logarithm of the calibrated OD values on the y-axis. Various plotting and statistical software can be used to assist in plotting the standard curve and calculating the concentrations of unknown samples. The four-parameter fitting method often yields better fitting results, while other methods such as linear fitting may also provide satisfactory fitting results. Analysis should be based on specific experimental data.

Experimental Data

1. Standard Curve Data

Fitting data to generate a standard curve for analysis of experimental data.



Standard Curve Graph

Concentration (ng/mL)	Absorbance		Mean Value	Calibration Value
1000	2.401	2.338	2.369	2.370
500	1.432	1.396	1.414	1.410
250	0.782	0.779	0.781	0.787
125	0.435	0.440	0.437	0.435
62.5	0.258	0.248	0.253	0.252
31.25	0.165	0.164	0.165	0.161
15.63	0.117	0.116	0.116	0.116
0	0.075	0.069	0.072	/

2. Sensitivity Test

The lower limit of detection for IL-1β/IL-1F2 is 11.46 pg/mL, calculated by averaging the OD values and standard deviation of zero wells tested 20 times.

3. Precision Testing

Intra-Assay Precision:

Three samples of known concentrations are tested eight times within the same microplate to evaluate intra-assay precision.

Inter-Assay Precision:

Three samples of known concentrations are tested 24 times across different microplates to evaluate inter-assay precision.

Project	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
	10	10	10	30	30	30
Mean Value	489.74	119.91	29.16	476.65	119.32	30.79
Standard Deviation	23.16	5.94	0.74	22.08	5.57	0.81
Coefficient of Variation (%)	4.7	5.0	2.5	4.6	4.7	2.6

4. Recovery Test

The recovery rates were determined by adding IL-1β/IL-1F2 at different levels to the samples. The recovery rates are as follows:

Sample Types	Average Recovery Rate (%)	Range (%)
Serum	94.6	81.2~107.6

Plasma	82.8	78.0~91.0
Cell culture supernatant	87.9	81.4~94.1

5. Dilution Linearity Test

Serum Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	103.0	98.1~106.9
1:04	105.5	101.3~106.6
1:08	111.3	105.2~123.3
1:16	94.5	89.5~98.3

Plasma Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	103.6	101.2~106.2
1:04	105.3	101.8~107.7
1:08	107.0	105.9~107.6
1:16	101.5	96.4~106.2

Cell Culture Supernatant Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	98.0	94.8~100.0
1:04	98.5	91.1~103.4
1:08	102.9	95.0~107.6
1:16	100.1	90.8~104.9

6. Sample Values

Applying this assay kit, samples from several healthy volunteers are tested, with unknown medication history of the volunteers.

Sample Types	Sample Number	Mean Value (ng/mL)	Sample Value (ng/mL)
Serum	10	n.d.	n.d.
Plasma	8	n.d.	n.d.
Cell Culture Supernatant	4	n.d.	n.d.

n.d.indicating that the sample concentration is below the detection range of 15.63 pg/mL

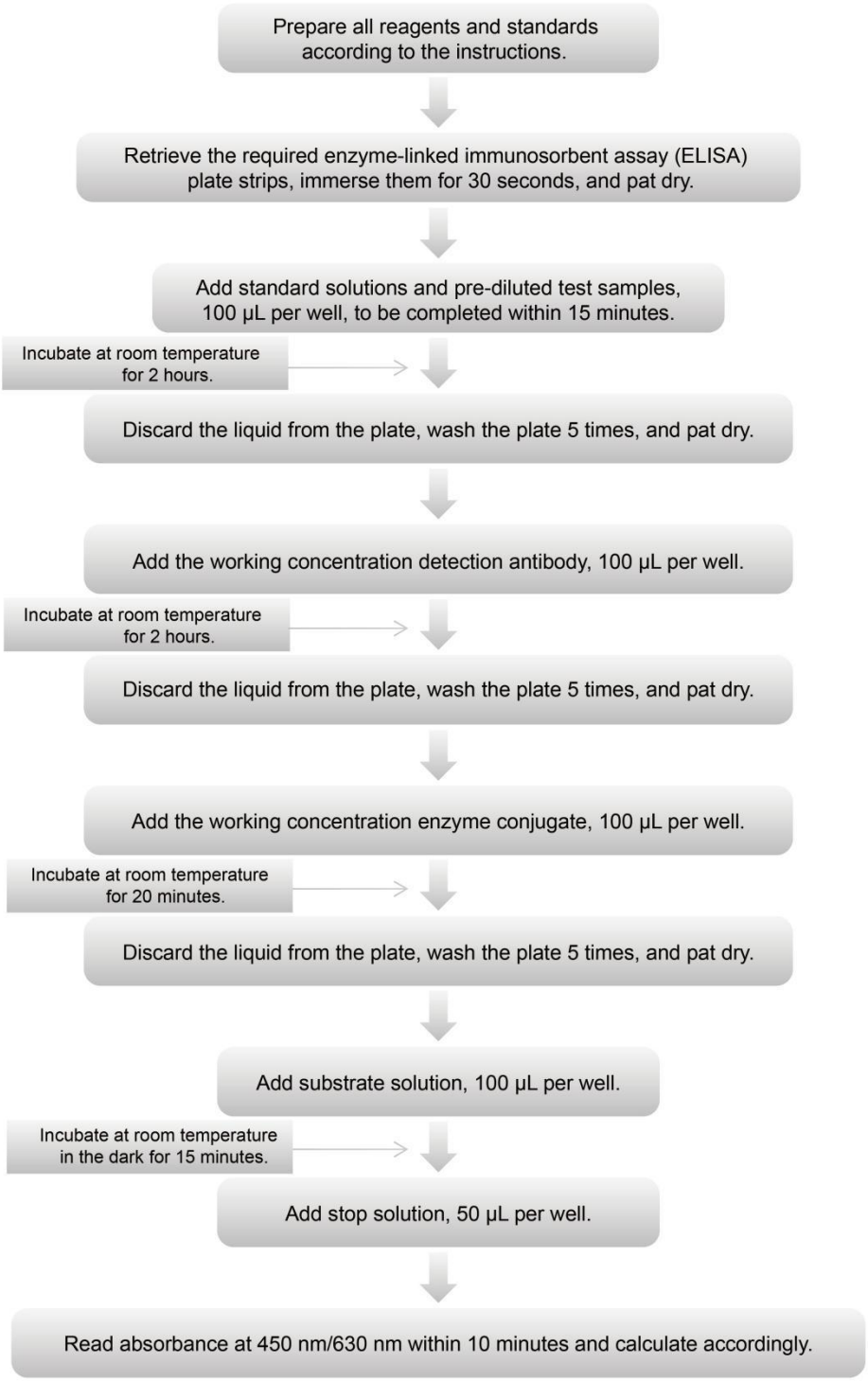
7. Specificity Determination

This assay kit specifically detects both natural and recombinant mouse interleukin 1β. Specific

evaluation of the following factors was conducted, and no significant cross-reactivity or interference was observed.

Recombinant human:		Recombinant Mouse:	
IL-1α	IL-10	IL-1α	IL-12
IL-1β	IL-12	IL-2	IL-13
IL-2	IL-13	IL-3	IFN-γ
IL-3	IFN-γ	IL-4	TNF-α
IL-4	TNF-α	IL-6	VEGF
IL-6	VEGF	IL-10	

Detection Schematic



Frequently Asked Questions

Issues	Causes	Solution
Poor standard curve	Inaccurate pipetting volumes	Check the pipette, calibrate it regularly, handle it carefully, tightly cap the tip while mixing thoroughly, and minimize foam formation.
	Inappropriate dilution methods	
Low color intensity	Insufficient incubation time	Allow sufficient incubation time, and replace samples and reconstituted standards overnight.
	Inadequate pipetting volumes or improper dilution	Calibrate pipettes and standardize operations.
High coefficient of variation (CV)	Improper washing of the ELISA plate	Use the correct washing procedure; if using a plate washer, check all ports for blockages.
	Contaminated washing solution	Prepare fresh washing solution.
Low sensitivity	Improper storage of the assay kit	Store according to the product component table.