Human IL-1β,IL-2,IL-6,IL-10,TNF-α,IFN-γ Multiplex ELISA KIT

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Item	Detection range	Sensitivity
IL-1β	1000-15.6pg/mL	5.2pg/mL
IL-2	1000-15.6pg/mL	5.2pg/mL
IL-6	1000-15.6pg/mL	5.2pg/mL
IL-10	1000-15.6pg/mL	5.2pg/mL
TNF-α	1000-15.6pg/mL	5.2pg/mL
IFN-γ	1000-15.6pg/mL	5.2pg/mL

Storage:-20℃(Short-term should be stored at 4℃,such as ~2 weeks).

Uses:Intended for the in vitro quantitative determination of Human IL-1β,IL-2,IL-6,IL-10,TNF-α,IFN-γ in liquid samples

Specifications:96T

Shelf Life:12 months when stored at -20℃

Human IL-1β,IL-2,IL-6,IL-10,TNF-α,IFN-γ Multiplex ELISA KIT COMPONENTS

PART	96 TESTS	48 TESTS	STORAGE
Single-analyte pre-coated plate	8×12	8×6	4/-20℃
Human IL-1β,IL-2,IL-6,IL-10,TNF-α,IFN-γ Premixed Standards	2 vial	1 vial	4/-20℃
Human IL-1β,IL-2,IL-6,IL-10,TNF-α,IFN-γ Premixed Biotinylated antibody (1:100)	1vial	1 vial	4/-20℃
Enzyme conjugate(1:100)	1vial	1 vial	4/-20℃
Enzyme diluent	1vial	1 vial	4/-20℃
Human IL-1β,IL-2,IL-6,IL-10,TNF-α,IFN-γ Premixed Antibody diluent	1vial	1 vial	4/-20℃
Premixed Standard diluent	1vial	1 vial	4/-20℃
Premixed Sample diluent	1vial	1 vial	4/-20℃
Washing buffer (1:25)	1vial	1 vial	4/-20℃
Color Reagent A	1vial	1 vial	4/-20℃
Color Reagent B	1vial	1 vial	4/-20℃

Color Reagent C	1vial	1 vial	4/-20℃
Manual	1 set	1 set	RT

NOTE

RT:Room temperature

Human IL-1β,IL-2,IL-6,IL-10,TNF-α,IFN-γ Standard:Lyophilized

Color Reagent A: Avoid light

OTHER SUPPLIES REQUIRED

1. Microplate reader (450nm detection wavelength filter,with optional 570nm or 630nm correction wavelength filters).
2. Washer (adjustable injection volume to ensure that each well receives 350μL without overflow).
3. Clean benches,biological safety cabinets,fume hoods.
4. High-precision single-channel pipette (range 0.5-10μL-20μL,20-200μL,200-1000μL).
5. High-precision multi-channel pipette (8 or 12,the range of 50-300μL of).
6. 37℃ incubator.
7. Low temperature centrifuge.
8. Refrigerators (4℃,-20℃,-86℃).
9. Analytical balance.
10. Scissors,tweezers,pliers,etc.
11. Plate mixer,low-frequency oscillator,etc.

ADDITIONAL MATERIALS REQUIRED

1. Centrifuge tubes (capacity of 1.5mL,5mL,etc.).
2. Disposable pipette tips (range of 0.5-10μL-20μL,20-200μL,200-1000μL).
3. Pure water or distilled water.
4. Coordinate paper.
5. Absorbent paper.

SAMPLE COLLECTION

1. Blood collection tubes should be both pyrogen- and endotoxin-free.
2. Hemolyzed or hyperlipidemic specimens are not recommended to be used.

3. Samples ultimately should appear clear and mostly transparent.All particulates should be removed through centrifugation.
4. If collected samples are not used immediately,they should be divided according to single usage quantities and stored frozen at -20-80℃,carefully avoiding repeated freeze-thaw cycling.
5. Sample dilution optimization is often necessary for proper sample resolution within the standard curve.Pre-experiments are always recommended to be performed prior to running the bulk of the samples,in order to determine if an optimizing dilution should be made to the samples.
6. It is recommended to collect sufficient samples for running multiple tests,since sometimes some users can experience issues that may result in a loss of data.
7. Always wear protective outerwear while collecting/handling samples (e.g.wearing gloves,coat,respirator,etc.) and be aware of all potential risks involved in handling your specimens.
8. Specimen processing should be conducted inside a properly-maintained biological safety cabinet.

SAMPLE PREPARATION

1. Serum: Place collected whole blood in refrigerator at 4℃ overnight.Then centrifuge for 10mins at 1000-3000rpm.Take supernatant and either test immediately or place samples at -20℃/-80℃(1-3 months) for storage.
2. Plasma: Take plasma where EDTA,sodium citrate,or heparin has been added as anticoagulant. Mix well.Centrifuge mixture for 10mins at 1000-3000rpm.Take supernatant and test immediately or place samples at -20℃ /-80℃ (1-3 months) for storage.
3. Tissue homogenate: Take tissue slices which have been washed in 0.01M PBS and then a tissue protein extraction reagent has been added according to proportion of 1g to 5-10mL and then mixed on ice bath. After sufficient homogenization,please then centrifuge for 10mins at 5000-10000rpm.Take supernatant for immediate testing,or place samples at -20℃/-80℃ (1-3 months) for storage.
4. Cell culture: Centrifuge for 10mins at 1000-3000rpm.Take supernatant for immediate testing,or place samples at -20℃/-80℃ (1-3 months) for storage.
5. For urine,ascites,cerebrospinal fluid,etc: Centrifuge for 10mins at 1000-3000rpm.Take supernatant for immediate testing,or place samples at -20℃/-80℃ (1-3 months) for storage.

NOTE

It is recommended that the user be well-informed regarding the general range of concentrations that are expected for their samples.We would suggest consulting the scholarly literature for references to similar samples' concentrations,and then to consider diluting your samples accordingly.

EXPERIMENTAL PREPARATION

1. Please remove Human IL-1β,IL-2,IL-6,IL-10,TNF-α,IFN-γ Multiplex ELISA Kit from refrigerator 20 mins in advance,and begin test once it has been brought to room temperature.
2. Dilute the concentrated washing buffer with double distilled water (1:25).Return unused quantity back to the box.
3. Standard:Add 1.0mL Standard Diluent to lyophilized standard vial and allow to sit for 30 mins.After the standard has completely dissolved,mix it slightly and mark with a

label on the tube.

4. Legend of standard sample dilution method: Take 7 clean tubes and label themwith ②,③,④,⑤,⑥,⑦,⑧ respectively.Add 300μL standard sample diluent intoeach tube.Pipette out 300μL diluent from tube ① to tube ②and mix well.Further Pipette out 300μL diluent from tube ② to tube ③,and mix well.Repeat steps above up to tube ⑦.Standard sample dilution in tube⑧is negative control.
5. Reconstituted standard stock solution cannot be reused.
6. Biotinylated Antibody: Remove the appropriate volume of Biotinylated Antibody solution for the quantity of wells intending to be assayed,and dilute with Antibody Diluent in a proportion of 1:100.This should be prepared 30mins in advance,and we would absolutely recommend not re-using for additional assaying.
7. Enzyme Conjugate: Remove the appropriate amount of Enzyme Conjugate solution for the quantity of wells intending to be assayed,and dilute with the Enzyme Diluent in a proportion of 1:100.This should be prepared 30mins in advance,and we would absolutely recommend not re-using for additional assaying.
8. Color Reagent: Prepare Color Reagent solution 30mins in advance by adding Color Reagent A and Color Reagent B by the proportion of 9:1.

Human IL-1β,IL-2,IL-6,IL-10,TNF-α,IFN-γ Multiplex ELISA KIT ASSAY PROCEDURE

1. Remove number of strips desired,and allow to acclimate to room temperature.The unused strips and desiccant should be placed back into the sealed aluminum foil bag and stored at 2-8℃.
2. Set aside blank wells (if measuring at dual-wavelength,the blank wells can be ignored)
3. Add mingchengAAA standards or samples to their corresponding wells (100μL for each well).Please remember that the 0danweiAAA well should be 100μL of Standard Diluent.Seal the wells/plate with the adhesive tape strip,and incubate at 37℃ for 90mins.
4. Prepare required quantity of Biotinylated mingchengAAA Antibody 30mins in advance.
5. Wash ELISA plate 2 times
6. Add prepared Biotinylated Antibody to each well (100μL per well).Seal reaction wells with adhesive tape strip,and incubate at 37℃ for 60mins.
7. Prepare required quantity of Enzyme Conjugate 30mins in advance.
8. Wash ELISA plate 3 times.
9. Add prepared Enzyme Conjugate to each well other than the blank wells (100μL for each).Seal the wells with the adhesive tape strip,and incubate at 37℃ for 30mins.
10. Wash ELISA plate 5 times.
11. Add 100μL of the prepared Color Reagent to individual wells (also into blank well),incubate protected from light at 37℃.When the coloration of the highest standards become darker,and the color gradient appears,the incubation can be stopped.The chromogenic reaction should be controlled to within 30 mins.
12. Add 100μL Color Reagent C to each individual well (also into blank well).Mix well.Read OD(450nm) within 10 mins.

Take the concentration values of standards as X- and OD readings as the Y-coordinates.Use a smooth line to connect each coordinate point of the standard values.The concentration of samples can be found by inputting the sample OD values into the line equation for the standard curve.It is recommended to employ professional curve software (e.g.curve expert 1.3) to analyze and compute the results.

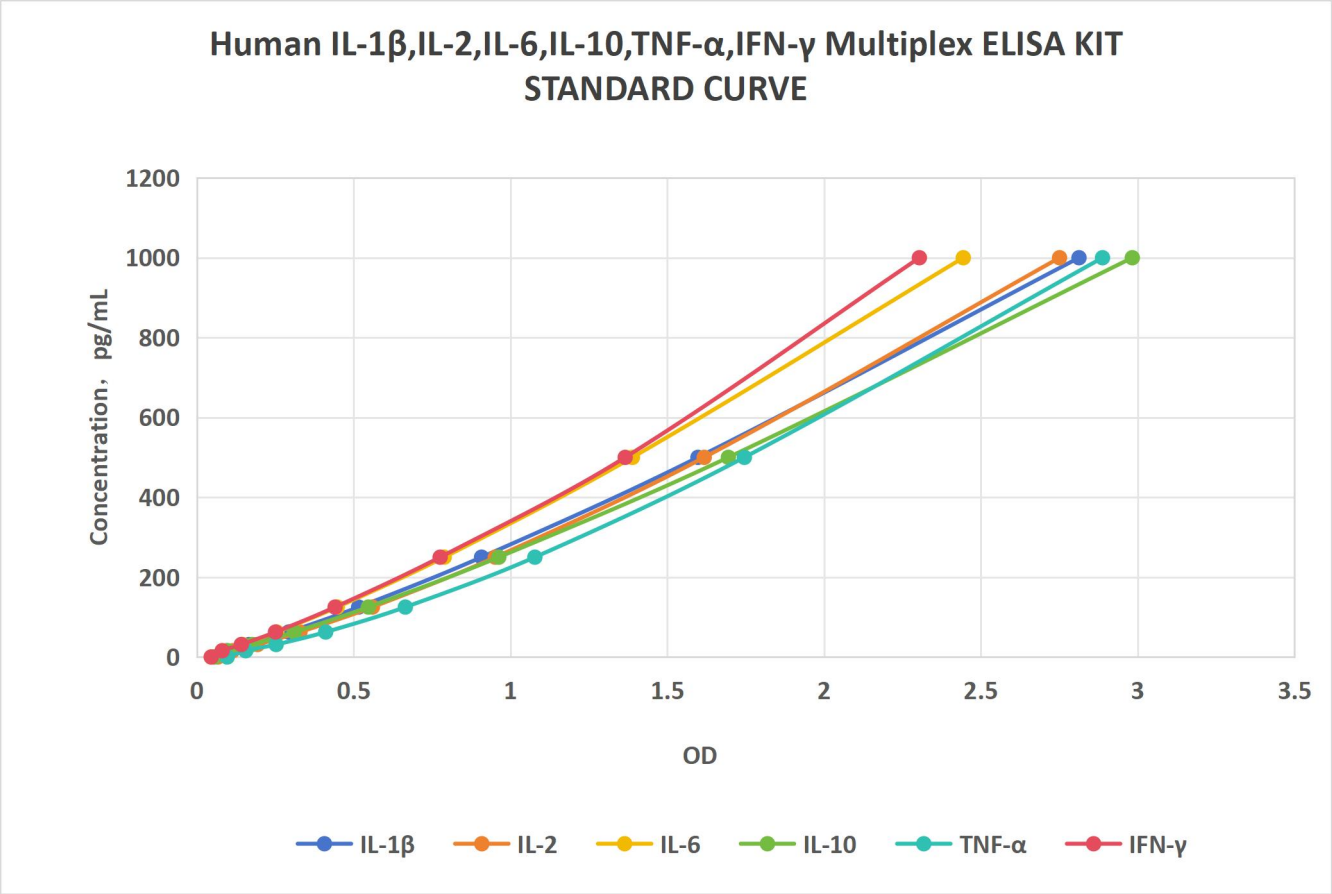
Human IL-1β,IL-2,IL-6,IL-10,TNF-α,IFN-γ Multiplex ELISA KIT TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

ABSORBANCE VALUE	Standard1	Standard2	Standard3	Standard4	Standard5	Standard6	Standard7	Standard8
	1000	500	250	125	62.5	31.25	15.625	0
IL-1 β	2.813	1.598	0.908	0.516	0.293	0.167	0.095	0.054
IL-2	2.751	1.618	0.952	0.560	0.329	0.194	0.114	0.067
IL-6	2.444	1.389	0.789	0.448	0.255	0.145	0.082	0.047
IL-10	2.983	1.695	0.963	0.547	0.311	0.177	0.100	0.057
TNF- α	2.828	1.746	1.078	0.665	0.411	0.253	0.156	0.097
IFN- γ	2.404	1.366	0.776	0.441	0.251	0.142	0.081	0.046

Standard Concentration:pg/mL

Human IL-1β,IL-2,IL-6,IL-10,TNF-α,IFN-γ Multiplex ELISA KIT STANDARD CURVE



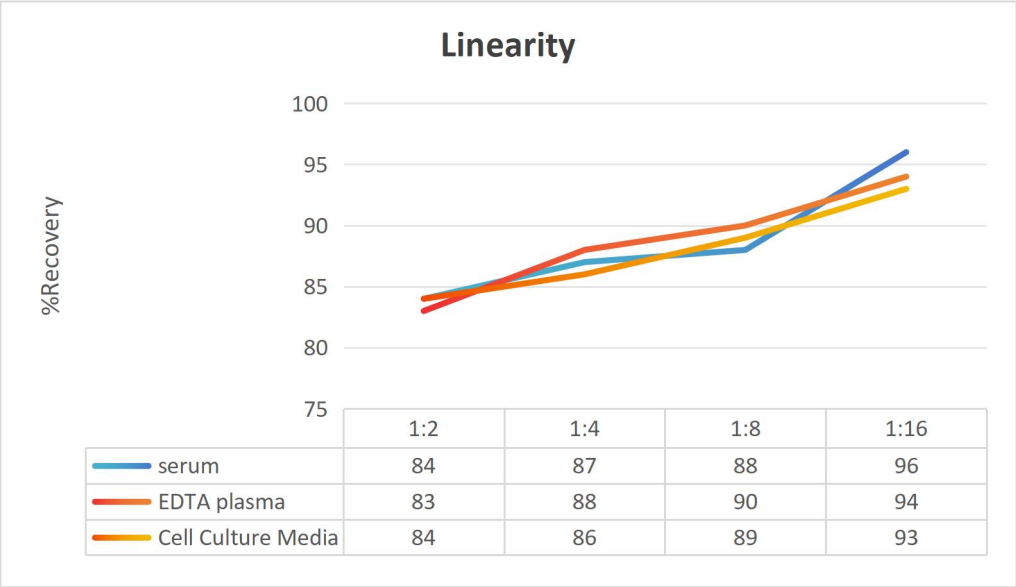
Recovery for Human IL-1β,IL-2,IL-6,IL-10,TNF-α,IFN-γ Multiplex ELISA KIT

The recovery of Human IL-1 β ,IL-2,IL-6,IL-10,TNF- α ,IFN- γ spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Matrix	Range %	Average % Recovery
serum(n=5)	83-108	95.5
EDTA plasma(n=5)	89-101	97
Cell Culture Media (n=5)	95-111	102

LINEARITY

To assess linearity of the assay, samples were spiked with high concentrations of Human IL-1 β ,IL-2,IL-6,IL-10,TNF- α ,IFN- γ and diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.



	1:2	1:4	1:8	1:16
serum	84	87	88	96
EDTA plasma	83	88	90	94
Cell Culture Media	84	86	89	93

PRECISION

Intra-Assay Precision (Precision within an assay)Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least two technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV%	6.6	5.5	5.2	8.3	7.5	6.9

RESULT DETERMINATION

1. The OD values of each sample and standard should be corrected by subtracting the OD value of the blank well.
2. Draw the standard curve manually: Plot the standard curve using the standard concentrations as the Y-axis and the corresponding OD values as the X-axis. Connect the plotted points with a smooth curve. The concentration of samples can be calculated by substituting the sample OD values into the standard curve equation. It is recommended to use professional curve-fitting software (e.g., Curve Expert 1.3) for result analysis and calculation.
3. If the sample OD value is higher than that of the highest-concentration standard in the standard curve, the sample should be diluted (or further diluted) and reassayed. Multiply the measured concentration by the dilution factor when calculating the final concentration.

NOTE

1. The re-dissolved standard cannot be stored again once prepared,so please do not attempt to re-freeze it once it has been reconstituted.
2. Due to shaking/inversion during transport,centrifuging of the tubes/bottles of the kit might be necessary to consolidate the material contained within.Tubes should be shaken manually or centrifuged for 1 min at 1000rpm to pool all material to the bottom.
3. Concentrated washing buffer might crystallize slightly.Use a water bath to help the dissolution during diluting process.The crystals must be totally dissolved when preparing the washing buffer.
4. The prepared standard is intended to only be a single-use aliquot,so please do not try to re-use the standard that has already been tested.Please use the second vial provided if you run the assay again.
5. Only use reagents/components that came directly with this kit.Do not mix batches/lots from other orders of this kit,or from different kits.
6. Ensure the reagents are well mixed.For the reagents in the microplate,adequate mixing is particularly important for accurate test results.It is recommended to employ a micro-oscillator (at the lowest frequency).If a micro-oscillator is not available,please slightly shake the microplate manually for 1 min,in a circular motion in order to make sure the wells are sufficiently mixed.
7. Please ensure the kit has been brought to room temperature prior to beginning the assay.
8. Standards are always recommended to be tested in duplicate or triplicate.
9. Place the unused microplate strips into the foil bag at 2-8℃ for storage (if you intend to use the strips within a relatively short timeframe).
10. The chromogen reagent is sensitive to light,therefore please avoid exposing to light.
11. Kits that have passed their expiration date should not be used.
12. When using dual-wavelength,the wavelengths should be set at 450nm and 630nm.
13. All the samples,washing buffer and wastes should be treated as biowaste.Color Reagent C is 1M sulfuric acid,so please pay close attention to safety when it is used.
14. Sample addition should always be done via pipette or similar instrument.Calibrate the instrument prior to running the assay in order to avoid experimental errors.Please add samples to the wells quickly,as it is recommended to control the sample addition time to less than ~5 mins.You might want to consider multi-pronged pipettes if this helps with the loading time.
15. Do not re-use the adhesive strips.Cut them to size if you only use part of the plate at a time.Always discard after use.

16. New standard curves should be made for every new run of the assay.If the observed concentrations of test samples are too high (OD value of the sample is higher than that of standard well maximum concentration),dilute by a certain factor,and correct for said factor in the end calculations.
17. Samples containing NaN3 cannot be tested due to NaN3 inhibiting the activities of horseradish peroxidase (HRP).
18. When washing plate via plate washer,the volume of buffer injected into each well should be slightly more than 350 μL.Make sure the sampling head is not jammed or blocked.Also,if washing by hand,please take care when using an absorbent material to remove excess water – make sure this absorbent material wasn't used to clean any of the other reagents to prevent contamination.
19. After the coloration reaction termination by Color Reagent C,please read OD within 10 mins.
20. If duplicate wells were performed,the mean value of the wells should be used.
21. Hemolyzed samples may cause false positive results,so we consider these samples to be incompatible with this kit.
22. During the assay,please try to control the humidity to ~60%.
23. We recommend regularly checking the thermostat and calibration in order to confirm the incubation temperature remains at a stable 37℃.