EcoPlex™ Human Inflammation 5-Plex Panel Kit

Catalog No: MPH001

Product size: 96T

Components

Component	Component Name	96T	Storage
MPH001A	1× Premixed antibody-conjugated beads	4.7 mL	2-8°C shading light
MPH001B	2× Premixed biotinylated detection antibodies	1.25 mL	2-8°C
MPH001C	2× Detection antibody diluent	1.25 mL	2-8°C
MPH001D	Lyophilized antigen standards	1 vial	2-8°C
MPH001E	1× SA-PE	2.5 mL	2-8°C shading light
MPH001F	10× Reading buffer	4 mL	2-8°C
MPH001G	10× Wash buffer	15 mL	2-8°C
MPH001H	SPB standard diluent	2.5 mL	2-8°C
MPH001I	SPB assay buffer	5 mL	2-8°C
	Plate Sealer	5 piece	
	Manual	1 copy	

Introduction

EcoPlex™ Human Inflammation 5-Plex Panel Kit is based on microsphere-based detection technology, which can simultaneously measure multiple target proteins and with only 15 μL sample usage. This kit is for detection of IL-1β, IL-6, IL-8, IL-18 and TNF-α in human serum, plasma, biological fluid and cell culture supernatant.

Detection Principle

EcoPlex™ kits utilize antibodies-conjugated beads which are different in size and mean fluorescent intensity of labeling dye to capture antigens in sample. After capture beads and sample incubation, biotin-conjugated detection antibodies are added to react with antigen-beads complexes, followed by a streptavidin-R-phycoerythrin (SA-PE) treatment. The fluorescent intensity of PE on each bead populations is quantified by flow cytometer with either a single 488nm laser or dual 488nm and 633/640nm lasers. The maximum emission of the bead classification dye is at 700nm. The concentrations of protein interested are determined by comparing the fluorescent intensities to those of standard curve, like sandwich ELISA data analysis.

Detection Sample Types

☑ serum ☑ EDTA plasma ☑ cell culture supernatants
☑ other biological fluids

Storage

Store at 2-8 ℃（protect from light）for 1 year.

Sample Collection and Materials Not Supplied

1) Serum

Allow samples to clot for 1 hour at room temperature or overnight at 2-8 ℃ before centrifugation for 20 min at 1000 ×g at 2-8 ℃. Collect the supernatant to carry out the assay.

2) Plasma

Collect plasma using EDTA-Na_2 as an anticoagulant. Centrifuge samples for 15 min at 1000 ×g at 2-8 ℃ within 30 min of collection. Collect the supernatant to carry out the assay.

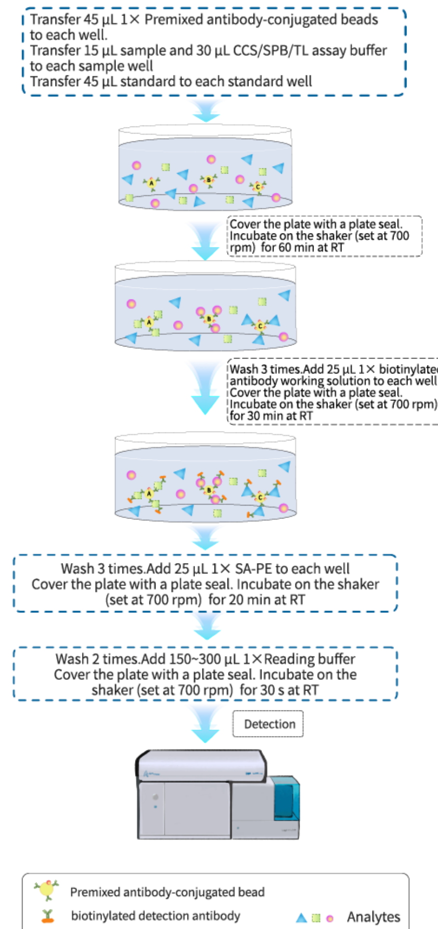
3) Cell culture supernatant or other biological fluids

Centrifuge samples for 20 min at 1000 ×g at 2-8 ℃. Collect the supernatant to carry out the assay.

4) Instrument

U-bottom 96-well transparent plates, vortex mixer, incubators for 96-well plate, centrifuge for 96-well plate, flow cytometry (with PE and APC or PE-CY5 or PE-CY7 detection channels

Experimental Operation



Diluent buffer preparation

1) 1×Reading buffer

Bring 10×Reading buffer to room temperature. Vortex 10× Reading buffer for 15 seconds. Dilute it with 9 times ddH_2O to prepare 1×Reading buffer. 1×Reading buffer can be stored at 2-8 ℃ for up to 3 months.

2) 1×Wash buffer

Bring 1×Wash buffer to room temperature. Vortex 10×Wash buffer for 15 seconds. Dilute it with 9 times ddH_2O to prepare 1×Wash buffer. 1×Wash buffer can be stored at room temperature for up to 3 months.

3) 1×biotinylated detection antibodies

Mix equal volume 2×Premixed biotinylated detection antibodies with 2×Detection antibody diluent. It can be stored at 2-8 ℃ for 24 hours.

Standards preparation

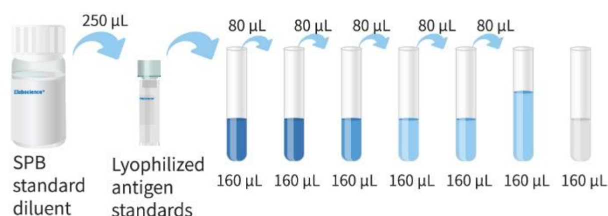
1) Centrifuge the Lyophilized antigen standard at 2000 ×g for 10 seconds
2) Add 250 µL of SPB standard diluent into the Lyophilized antigen standard
3) Vortex gently for 15 seconds
4) Keep standard on ice for 5-10 minutes
5) Vortex gently for 15 seconds before the serial dilution

NOTE：Preparing standards and standards serial dilution within 2 hours before sample and beads incubation. Don't freezing, thawing and reuse of the re-constituted and diluted standard. Please order extra vials of antigen standards if running a partial plate.

Serial dilution preparation

Take 7 EP tubes, add 160 µL of SPB standard diluent to each tube. Pipette 80 µL of the redissolved standard working solution to the first tube and mix. Pipette 80 µL of the solution from the former tube into the latter one according to this step. The illustration on the next page is for reference. The last tube is regarded as a blank. Don't pipette solution into it from the former tube. Keep the standards on ice until use.

NOTE：Mix diluent by pipetting up and down 6–8 times after each addition. Change pipette tips after each addition to avoid contamination from one concentration to the other. If the concentration of cytokines in sample is very low (less than 10 pg/mL)， we recommend addition 1-2 standard dilution， e.g. Standard 8 (1:2187) and Standard 9 (1:6561). Standard 1 may be omitted when measuring these samples.



Assay procedure

1) Label 96-well plate with Standard and Sample name. For each standard and sample, 1-2 repeats are recommended.
2) Vortex Premixed antibody-conjugated beads for 15 seconds. Add 45 µL beads to each well, the remaining beads are stored away from light at 2-8°C.
3) Centrifuge the 96-well plate at 300 ×g for 5 minutes, discard supernatant.
   NOTE：Discard supernatant by throwing. Don't repeat discarding to avoid loss of beads.
4) Add 15 µL sample and 30 µL SPB assay buffer to each sample well. Add 45 µL standard to each standard well. If sample contains very low level cytokines (less than 20 pg/mL), add 45 µL sample to each well.
5) Cover the plate with a plate seal. Incubate on the shaker (set at 700 rpm) for 60 minutes at room temperature. Upon incubation, protect the plate from light.
6) Remove the plate seal. Centrifuge the 96-well plate at 300 ×g for 5 minutes. Discard supernatant.
7) Add 100 µL 1×Wash buffer to each well. Centrifuge the 96-well plate at 300 ×g for 5 minutes and discard supernatant. Wash three times.
8) Add 25 µL 1×biotinylated detection antibodies working solution to each well.
9) Cover the plate with a plate seal. Incubate on the shaker (set at 700 rpm) for 30 minutes at room temperature. Upon incubation, protect the plate from light.
10) Remove the plate seal. Centrifuge the 96-well plate at 300 ×g for 5 minutes. Discard supernatant.
11) Add 100 µL 1×Wash buffer to each well. Centrifuge the 96-well plate at 300 ×g for 5 minutes and discard supernatant. Wash three times.
12) Add 25 µL 1× SA-PE to each well.
13) Cover the plate with a plate seal. Incubate on the shaker (set at 700 rpm) for 20 minutes at room temperature. Upon incubation, protect the plate from light.
14) Remove the plate seal. Centrifuge the 96-well plate at 300 ×g for 5 minutes. Discard supernatant.
15) Add 100 µL 1×Wash buffer to each well. Centrifuge the 96-well plate at 300 ×g for 5 minutes and discard supernatant. Wash two times.
16) Add 150-300 µL 1×Reading buffer to each well.
17) Cover the plate with a plate seal. Incubate on the shaker (set at 700 rpm) for 30 seconds at room temperature before reading on a flow cytometer.
18) Remove the plate seal. Read on a flow cytometer
    NOTE：The assayed plate can be stored at 2-8°C for up to 16 hours. The plate should be sealed with a plate seal and protected from light.

Setting up Flow Cytometers

1. Fluorescence channels

The maximum emission of the bead classification dye is at 700 nm. It can be detected on PE-Cy5 channels or PE-Cy7 channels of flow cytometers with blue (488 nm) excitation. It can also be detected on APC channel with red (633 or 640nm) excitation. The reporter dye of the assays is PE and can be detected on the PE channel with blue (488 nm) excitation.

2. Preparing setup beads

Vortex Premixed antibody-conjugated beads for 15 seconds. Add 45 µL beads to a well or tube. Add 100-200 µL 1×reading buffer to the well/tube.

3. Setting up template

1) Open a new experiment. Create the following plots and histograms:
   A dot plot with FSC (X-axis) and SSC (Y-axis) in linear display mode.
   Two dot plot with PE (X-axis) and PE-Cy5/PE-Cy7/APC (Y-axis) in Log display mode.
2) Run setup beads. Adjust FSC and SSC gates to ensure the bead populations are on scale. Create gate for two different size bead populations (Figure 1).
3) Apply Gate 1/2 to the PE VS PE-Cy5/PE-Cy7/APC dot plot. Adjust PE-Cy5/PE-Cy7 PMT voltage to ensure all the bead populations are clearly separated on both dot plots.
4) Run Standard 1 to make sure all the bead populations on the PE-axis are on scale. If necessary, adjust PE PMT voltage.
   NOTE: If re-setting PE PMT voltage, re-run the Blank and make sure the dimmest bead population is still visible on the PE VS PE-Cy5/PE-Cy7/APC dot plots.
5) Apply proper PE/PE-Cy7 and PE/PE-Cy5 color compensation so that the bead populations are in a horizontal position (Figure 2).
6) Create gates for different bead populations according to fluorescent intensity of classification dye. Apply gates to a PE histograms.
7) Save the template.

NOTE: The template is provided for reference only.

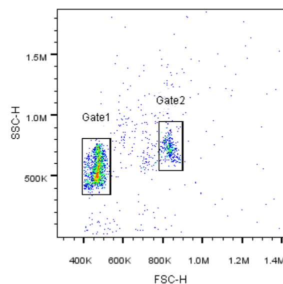


Figure 1

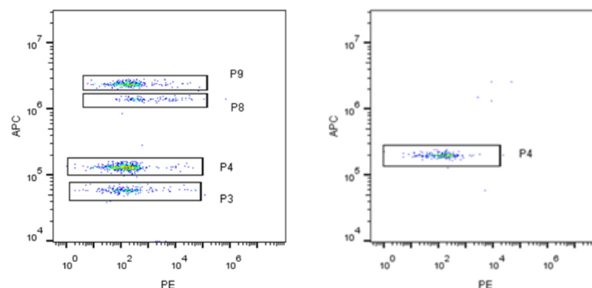


Figure 2

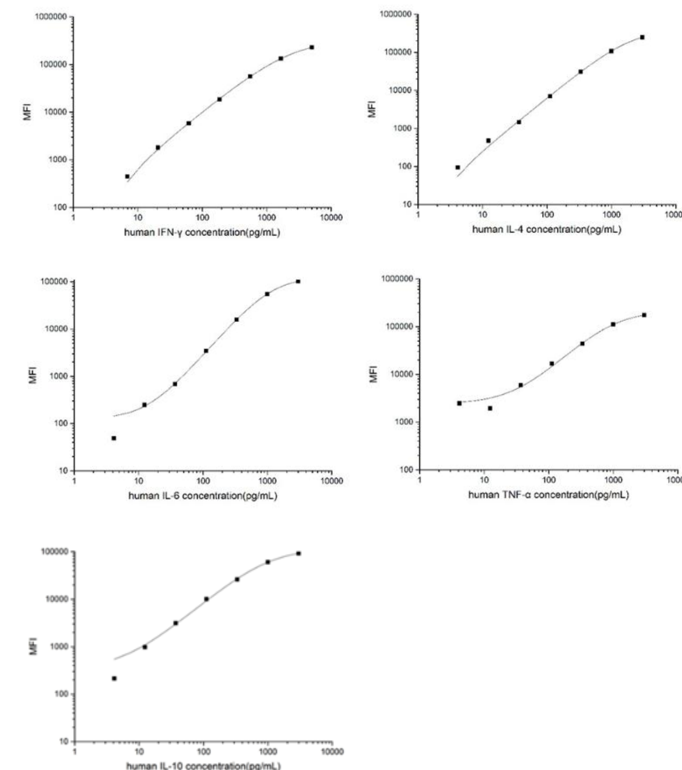
4. Analyzing the samples

1) Vortex the samples on the shaker (set at 700 rpm) for 30 seconds at room temperature.
2) Open the template and run samples. Acquire 200 events for each bead population of the larger beads (Gate 2). For example, if there are 4 bead populations in Gate 2 (larger beads), acquire 4×200 = 800 events per sample. The amount of each bead population should not less than 200 events.

5. Results calculation

1) Average the duplicate readings for each standard and samples, then subtract the average zero standard Median fluorescence intensity. Plot a four parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and Median fluorescence intensity on the y-axis.
2) If the Median fluorescence intensity of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Typical data



NOTE: Typical standard curve and data is provided below for reference only.

Performance Parameters

Analytes	Gates	LLOQs (pg/mL)	ULOQs (pg/mL)	Sensitivity (pg/mL)
IL-1β	Gate1P2	<10	>5000	<5
IL-6	Gate1P4	<10	>5000	<5
TNF-α	Gate1P6	<2	>1000	<1
IL-8	Gate2P2	<1	>1000	<1
IL-18	Gate2P8	<10	>5000	<5

Standard dose recovery：70-130%

Intra-assay CV：<10%

Inter-assay CV：<20%

Cross-reactivity of analytes in the Panel：Negligible

Note: The concentrations of standards may vary from batch to batch and therefore will not be labeled uniformly.

Troubleshooting

Symptoms	Causes	Comments
Low bead number in samples	Not transfer enough beads	Vortex stock beads suspension before adding beads to well
	Beads lose during wash step	Gently discard supernatant.
	Beads aggregate	Shake plates at 700 rpm for 30 seconds prior to acquisition or re-suspend the beads in a well by pipetting up and down 6–8 times prior transfer to tube.
High Background	Well-to-well contamination	Change pipette tips after every transfer. Remove plate seal carefully and avoid contents from one well to mix with another.
Low sensitivity	Standard not reconstituted well	Standard should be incubated on ice for 5-10 minutes after the addition of standard diluent.
Low sample detection rate	Low cytokine expression level in samples	Add samples to each well without dilution.

Cautions

1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. Vortex stock beads suspension before adding beads to well.
4. Change pipette tips after every transfer.
5. Remove plate seal carefully and avoid contents from one well to mix with another.
6. Avoid creating bubbles when pipetting.
7. Multi-channel pipette is recommended for reagent additions to achieve optimal assay precision.
8. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the flow cytometry. Please read the instructions carefully and adjust the instrument prior to the experiment.
9. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions.