Human TNF-α/IFN-γ/IL- 1β/IL-2/IL-4/IL-5/IL-6/IL-8/IL-10/IL-12p70/IL-17/IFN-α Cytokine Kit (Flow Cytometry Multiplex Bead Assay)

Cat #: orb1975799 (manual)

Reagents And Materials Provided

	Items	Components	48T	96T
A	Capture Bead Mixture	Microspheres conjugated with antibodies against human TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, and IFN-α.	2.5mL	5mL
B	Detection Antibody Mixture	Biotinylated detection antibodies against human TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, and IFN-α.	5mL	10mL
C	Standard	Lyophilized recombinant cytokine proteins of human TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, and IFN-α.	1	2
D	PE-Labeled Streptavidin	SAV-PE	5mL	10mL
E	Blank Microspheres	Microspheres	0.3mL	0.3mL
F	Standard/Sample Diluent	-	16mL/bottle×1 bottle	16mL/ bottle×2 bottle
G	20×Wash Buffer	-	25mL/ bottle×1 bottle	25mL/ bottle×1 bottle
H	Microplate	-	96-well plate × 1 plate	96-well plate × 1 plate
I	Plate Sealer	-	4 pieces	4 pieces

Storage: Store at 2–8°C, protected from light. The shelf life is 18 months. After opening, store at 2–8°C in the dark and use within 30 days.

Introduction

This kit uses CBA (Cytometric Bead Array) multiplex flow cytometry technology. Different microsphere populations are encoded with distinct fluorescence intensities, and each microsphere population is coated with specific antibodies against TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, and IFN-α. After incubation with the test samples, the coated microspheres specifically bind to the corresponding cytokines in the samples.

Biotin-labeled detection antibodies are then added to form immune complexes consisting of antibody-coated microspheres, cytokines, and detection antibodies. Subsequently, phycoerythrin (PE)-labeled streptavidin is added to bind with biotin. The fluorescence intensity of each analyte is measured using a flow cytometer. The fluorescence intensity is proportional to the concentration of TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, and IFN-α in the sample. By combining the fluorescence

signals with the standard curves generated from the twelve cytokine standards, this kit enables simultaneous quantitative detection of TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, and IFN-α in a single sample, providing support for evaluating immune function status.

Intended Use

For the detection and quantification of TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, and IFN-α expression levels in human serum, plasma, and cell culture supernatants.

Compatible Instruments

This product is compatible with most commercially available flow cytometers equipped with PE and APC detection channels.

Sample Requirements

Serum, plasma, and cell culture supernatant samples should preferably be tested within 4–6 hours after collection. If immediate testing is not possible, samples should be stored at −20°C. Avoid repeated freeze-thaw cycles.

Assay Procedure

1. Preparation of Wash Buffer (1×)

Invert the bottle labeled 20× Wash Buffer several times to mix thoroughly. Add 25 mL of 20× Wash Buffer to 475 mL of distilled water and gently mix to avoid foaming. Store at 2–8°C until use.

2. Standard Preparation

(Prepare eight 1.5 mL EP tubes and label them as tubes 1, 2, 3, 4, 5, 6, 7, and 8.)

2.1 Before use, reconstitute the standards for each cytokine according to the table below using standard diluent. Mix thoroughly and allow the solution to stand at room temperature for 10 minutes. Take 16.67 µL from each tube and combine them into a single tube. This tube will be used as the highest concentration standard (Tube 1: 5000 pg/mL).

Standard	Qty（vial）	Content/vial	Volume of diluent added (µL)
human TNF-α	1	1000pg	16.67
human IFN-γ	1	1000pg	16.67
human IL-2	1	1000pg	16.67
human IL-4	1	1000pg	16.67
human IL-6	1	1000pg	16.67
human IL-5	1	1000pg	16.67
human IL-10	1	1000pg	16.67
human IL-12P70	1	1000pg	16.67

human IL-1β	1	1000pg	16.67
human IL-8	1	1000pg	16.67
human IL-17	1	1000pg	16.67
human IFN-α	1	1000pg	16.67

2.2 Add 150 µL of Standard/Sample Diluent to each of the remaining seven EP tubes (tubes 2–8) to prepare the serially diluted standards.

Tube	Add from last tube (µL)	Standard/Sample Diluent（µL）	Dilution Ratio (Compared to Standard 1)	Concentration (pg/mL)
1	-	-	-	5000
2	50	150	1:4	1250
3	50	150	1:16	312.5
4	50	150	1:64	78.13
5	50	150	1:256	19.53
6	50	150	1:1024	4.88
7	50	150	1:4096	1.22
8	-	150	-	0

2.3 Transfer 50 µL of standard from tube 1 to tube 2 and mix thoroughly. Then transfer 50 µL from tube 2 to tube 3 and mix thoroughly. Continue serial dilution in the same manner through tube 7.

3. Sample Preparation

For serum or plasma samples, it is recommended to dilute the samples 2-fold using Standard/Sample Diluent.

Sample	Dilution Factor 1:1	Dilution Ratio
Serum or plasma samples	25µL Sample+25µL Standard/Sample Diluent	2

4. Assay Procedure

•Before the experiment, remove all reagents and allow them to equilibrate to room temperature.

•The CBA 96-well plate should be properly secured onto the magnetic plate and fit tightly with the magnetic plate.

•Protect the plate from light during incubation.

•Before starting the experiment, it is recommended to run 50 µL of blank microspheres on the flow cytometer to adjust the voltage settings and identify each microsphere population. Ensure that the number of microsphere populations matches the instructions before proceeding with the experiment.

4.1 Transfer **50 µL** of each serially diluted standard from tubes 1–8 into the corresponding wells of the 96-well plate. Tube 8 contains standard/sample diluent.

4.2 Add **50 µL** of diluted serum or plasma samples into the remaining sample wells.

4.3 Vortex the microsphere mixture for 30 seconds. Add **50 µL** of microsphere mixture to each well. The final volume in each well should be **100 µL/well**. During microsphere addition, vortex the microsphere tube regularly to prevent microsphere sedimentation. It is recommended to vortex and mix the microspheres once after every 4 wells are added.

4.4 Place the 96-well plate on a shaker and mix at 450 rpm for 2 minutes.

4.5 Incubate the 96-well plate at 37°C for 30 minutes.

4.6 Place the 96-well plate on the magnetic plate for 5 minutes to allow microsphere separation, then remove the supernatant. When removing the supernatant, first discard the liquid from the wells, then invert the plate onto absorbent paper. The 96-well plate should remain secured on the magnetic plate during this process.

4.7 Remove the 96-well plate from the magnetic plate and add **200 µL** of wash buffer to each well.

4.8 Place the 96-well plate back onto the magnetic plate for 5 minutes and remove the supernatant.

4.9 Remove the 96-well plate from the magnetic plate and add **100 µL** of detection antibody to each well.

4.10 Incubate at 37°C for 30 minutes.

4.11 Repeat **steps 4.6–4.8**.

4.12 Remove the 96-well plate from the magnetic plate and add **100 µL** of phycoerythrin (PE)-labeled streptavidin to each well.

4.13 Incubate at 37°C for 15 minutes.

4.14 Repeat steps **4.6–4.8**.

4.15 Add **200 µL** of wash buffer to each well to resuspend the samples, then analyze using a flow cytometer. Before acquisition, ensure that the PE voltage is properly adjusted to ensure that the microsphere populations fall within the detection range of the PE channel.

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5. Flow Cytometry Analysis

5.1 Microsphere Population Distribution:

Specificity	Microsphere Location	Assigned Microsphere Region
human TNF-α	L1	R1
human IFN-γ	L2	R1
human IL-2	L3	R1
human IL-4	L4	R1
human IL-6	L5	R1
human IL-10	L6	R2
human IL-12P70	L7	R2
human IL-1β	L8	R2
human IL-8	L9	R2
human IL-17	L10	R2
human IFN-α	L11	R3
human IL-5	L12	R3

Note: Different fluorescent dyes with different intensities are used to stain the inside of the microspheres.

5.2 Template Setup (Reference):

(1) Establish a density plot template with FSC-H as the X-axis and FSC-A as the Y-axis. Set gates to exclude debris and aggregated microspheres, as shown in Figure 1.

(2) Establish a linear density plot template with FSC-H as the X-axis and SSC-H as the Y-axis. Define the mixed microsphere populations as regions R1, R2, and R3, as shown in Figure 2.

(3) Establish two logarithmic density plot templates with PE as the X-axis and APC as the Y-axis. Display the microspheres within the R1, R2, and R3 gates, respectively, so that the microsphere populations are clearly distributed on the scatter plots and the PE fluorescence intensity of each factor can be displayed. See Figures 3, 4, and 5.

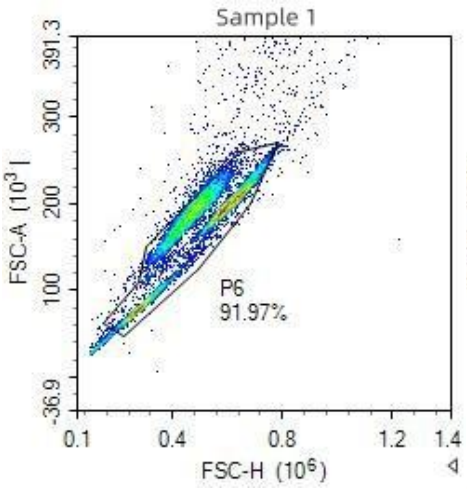

Figure 1

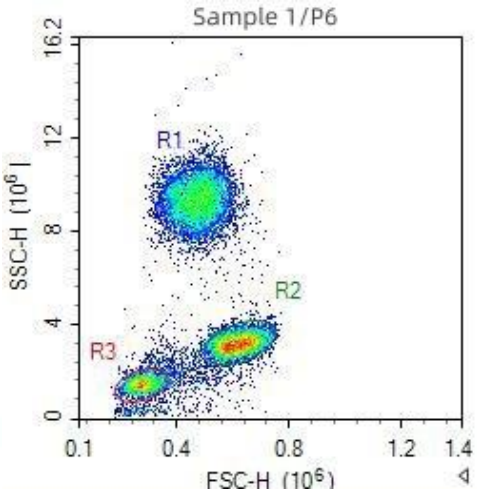

Figure 2

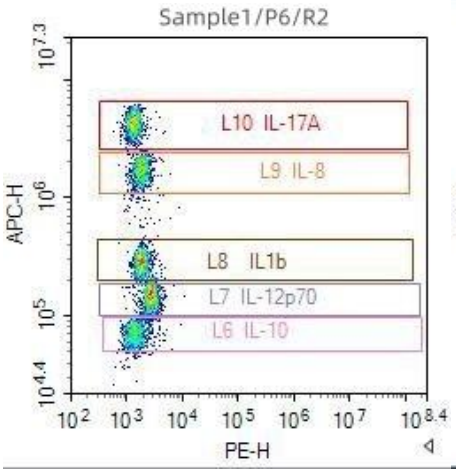

Figure 3

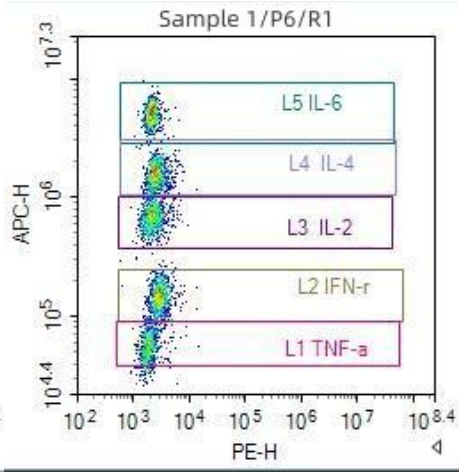

Figure 4

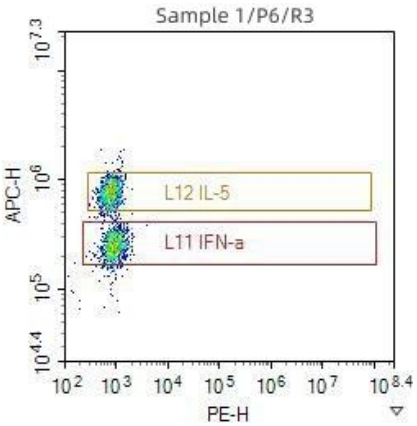

Figure 5

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5.3 Analysis of Sample Detection Results

All standards and samples are sequentially analyzed using the flow cytometer. For each microsphere population, at least 100 microspheres should be acquired. Standard curves are generated using the serially diluted standards, and sample concentrations are calculated based on the standard curves.

6. Software Analysis

Analyze data using FCAP software.

Reference Range:

A normal reference range was established by testing 150 healthy individuals using a ten-cytokine detection kit (flow cytometry fluorescence-based method):

IL-1β ⩽ 3.4 pg/mL, IL-2 ⩽ 11.4 pg/mL, IL-4 ⩽ 12.9 pg/mL, IL-6 ⩽ 20.1 pg/mL, IL-5 ⩽ 8.57 pg/mL, IL-8 ⩽ 15.71 pg/mL, IL-10 ⩽ 5.9 pg/mL, IFN-γ ⩽ 15.1 pg/mL, TNF-α ⩽ 6.1 pg/mL, IL-12p70 ⩽ 10.18 pg/mL, IL-17 ⩽ 8.57 pg/mL, and IFN-α ⩽ 6.1 pg/mL.

(Due to differences in environmental conditions, gender, age, and other factors, these values are provided for reference only.)

Interpretation of Detection Results

1.
If the measured cytokine concentration in the sample falls within the detection range shown in Table 3, the result is considered valid and can be directly reported.
If the result exceeds the detection range, dilute the sample appropriately using Standard/Sample/Microsphere Diluent and repeat the test. If the sample concentration is below the lower detection limit or not detected, report the result as ⩽ the minimum detectable value.

Specificity	Detection Range
human TNF-α	2.44pg/mL-5000pg/mL
human IFN-γ	19.53pg/mL-5000pg/mL
human IL-2	2.44pg/mL-5000pg/mL
human IL-4	1.22pg/mL-5000pg/mL
human IL-6	19.53pg/mL-5000pg/mL
human IL-5	1.22pg/mL-5000pg/mL
human IL-10	1.22pg/mL-5000pg/mL
human IL-12P70	4.88pg/mL-5000pg/mL
human IL-1β	9.77pg/mL-5000pg/mL
human IL-8	2.44pg/mL-5000pg/mL
human IL-17	4.88pg/mL-5000pg/mL
human IFN-α	2.44pg/mL-5000pg/mL

2. Recommendation:
Personnel responsible for data interpretation and report generation should receive formal technical training.

Limitations of the Assay Method:
Improper sample collection, transportation, storage, processing, or incorrect instrument settings may result in inaccurate detection results.

Product Performance Specifications

1. Appearance and Physical Characteristics
All kit components should be complete and intact. There should be no leakage of liquid reagents. The outer packaging should be intact without damage, and labels should be clear and easy to identify.

2. Fill Volume
The volume of each component should meet the specified requirements and should not be lower than the labeled amount.

3. Accuracy
When testing samples with known concentrations using this kit, the relative deviation of the measured results should be within ±15%.

4. Reproducibility
When this kit is used to detect TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, and IFN-α, the coefficient of variation (CV) should be ≤15%.

Precautions:

1. This kit is intended for research use only and is not for clinical diagnosis.
2. Experimental samples, quality controls/standards, and laboratory waste should be treated as potentially infectious materials and handled according to applicable regulatory safety procedures.
3. Incorrect calibration of the flow cytometer, improper compensation for fluorescence spillover, or inaccurate definition of detection regions (gating) may lead to incorrect results. Please refer to the instrument operating procedures for calibration and ensure that the instrument is in optimal condition before use.
4. This product contains fluorescent reagents. Avoid direct contact with skin or contamination of food. Gloves must be worn during operation.
5. After reconstitution, the standards should be used within 10 hours.
6. The Capture Bead Mixture must be thoroughly mixed by vibration before use.
7. To ensure the quality of fluorescence detection, all steps involving detection antibodies should be performed while protected from light.
8. Do not mix reagents from different lot numbers. Use the reagents within their specified expiration period.