# Aptplex™ Human Th1/Th17 12-Plex Panel

Catalog No：MPA011 Aptplex™ Human COVIDovid-19 8-Pplex Panel

Catalog No：MPA001

Product size：96 T

## Intended Use

Aptplex™ Human Th1/Th17 12-Plex Panel Human COVID-19 8-plex Panel is based on multiplex bead-based technology, enabling simultaneous quantification of multiple analytes from a single sample. This kit is suitable for the *in vitro* quantitative detection of concentrations of the following cytokines in human serum, plasma, cell culture supernatants, and other biological fluids:

IL-17A , IL-6 , TNF-α , IFN-γ , IL-12p70 , IL-10 , CCL5(RANTES) , CXCL10(IP-10) , IL-1β , IL-18 , sCD54(ICAM-1) , IL-2RA(sCD25)IL-6, IL-1β, TNF-α, IFN-γ, CXCL10 (IP-10), CCL2 (MCP-1), IL-8 (CXCL8), and IL-10.

## Components

| Component | Component Name | 96T | Storage |
|---|---|---|---|
| MPA0011A | Premixed Antibody-Conjugated Beads | 2.4 mL×2 | 2-8°C shading Protected from light |
| MPA0011B | Biotinylated Detection Antibodies | 4.8 mL×2 | 2-8°C |
| MPA0011C | SA-PE (ready to use) | 4.8 mL×2 | 2-8°C shadingProtected from light |
| MPA0011D | Lyophilized Standard | 2 vials | 2-8°C |
| MPA01101E | Assay Buffer | 5 mL×1 | 2-8°C |
| MPA0011F | Wash Buffer | 30 mL×2 | 2-8°C |
| | Plate Sealing Filmer | 5 pieces | |
| | Manual | 1 copy | |

## Introduction

Aptplex™ Human Covid-19 8-Plex Panel is based on multiplex bead-based technology, enabling simultaneous quantification of multiple analytes from a single sample. This kit is suitable for the in vitro quantitative detection of concentrations of IL-6 ; IL-1β ; TNF-α ; IFN-γ ; CXCL10(IP-10) ; CCL2(MCP-1) ; IL-8(CXCL8) ; IL-10 cytokines in human serum, plasma, cell culture supernatants, and other biological fluids.

## Detection Principle

The Aptplex™ assay is a multiplex bead based immunoassay that utilizes uses antibody-conjugated magnetic beads with distinct mean fluorescence intensities to capture target antigens from samplessimultaneously. When selected capture beads are mixed and incubated with samples containing specific Each targets antigen is recognized by the a specific capture antibodyies, each analyte binds to its corresponding on the bead and . Subsequently,binds with a corresponding biotinylated detection antibody to form a ies are added, each of which binds to a specific analyte, forming a "sandwich" complex of bead-analyte-detection antibody "sandwich complex". Streptavidin-phycoerythrin (SA-PE) is then introduced to binds to the biotinylated detection antibodies producing a fluorescent signal proportional to . The fluorescence intensity specific tothe amount of each analyte. The fluorescence of each bead is measured using flow cytometry. By and correlated ing the results with a standard curve generated from antigen standards, quantitative detection of target proteins in samples is achieved.to determine analyte concentrations.

## Detection Sample Types

☑ sSerum ☑ EDTA Pplasma

☑ cCell culture supernatants ☑ oOther biological fluids

## Storage

Store protected from light at 2-8 ℃ with a shelf life of 12 months. After opening, protect from light and store at 2-8 ℃, stable for up to 30 days. Reconstituted Standard should be used within 24 hours.

| Material | Storage Conditions | Stability / Notes |
|---|---|---|
| Unopened kit | 2–8 °C, protected from light | 12 months |
| Opened kit | 2–8 °C, protected from light | Up to 30 days |
| Reconstituted standard | 2–8 °C, protected from light | Use within 24 hours |

## Sample Collection and Materials Not Supplied

**1) Serum**

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

**3) Cell culture supernatant or other biological fluids**

批注 [S1]: Please confirm the correct trademarked product name and its capitalization, should it be 'Aptplex', 'APTPlex', or 'APTplex'? Consistent use of the correct capitalization is important not only for clarity and professionalism in the manual but also to accurately reflect the official trademark as registered by the company. And once you confirm it, revise it throughout the document

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批注 [S3]: A table format makes storage conditions clear, concise, and easy to reference.

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批注 [S2]: The current Detection Principle reads more like a step-by-step protocol. It should be revised to focus on the scientific mechanism of the assay rather than procedural details, making it concise and clear for the manual. I strongly suggest to keep my version.

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

### 4) Instrument Materials Not Supplied

- U-bottom 96-well transparent plates.
- vVortex mixer.
- iIncubators suitable for 96-well plate.
- mMagnetic separator.
- fFlow cytometery (with PE , APC and APC/Cy7 detection channels).

## Standard Preparation Procedure

1. Prepare eight 0.6 mL microcentrifuge tubes as standard dilution tubes and label them 0--7. Leave tube 7 empty. Add 150 µL of **Assay Buffer** to tubes 0--6.

2. **Reconstitute the lyophilized standard**

- The standard is supplied as a lyophilized powder. Before reconstitution, bBriefly centrifuge the vial tube at 500 × g for 10 seconds to collect the powder at the bottom of the tube.
- Add 500 µL of **Assay Buffer** to the vial. Let it stand for 5 min.
- , then gently pipette up and down 2-3 times to ensure theMix gently until the lyophilized standard is completely dissolved. and mixed.
- Transfer the entire solution to tube 7. This is the highest concentration standard solution.

3. **Prepare serial dilutions**

- PipetteTransfer 50 µL from tube 7 into tube 6 and mix thoroughly (1:3.2 dilution). This is the 1:3.2 dilution standard solution.
- Serially dilute the standard solution by transferring 100 µL from each tubes 6 to 2 in to the next respectivelower tube (tubes 5 to 16 → 5 → 4 → 3 → 2 → 1), mixing thoroughly at each step. This creates the standard solutions for tubes 5-1.
- The Assay Buffer in tTube 0 contains Assay Buffer only and serves as the zero concentration standard.

Note: The specific exact concentration of the highest concentration standard solution may vary. Please refer to is indicated on the standard vial label for details.

## Procedure

1. **Bead and Sample incubation**

1. Add 50 µL of **Premixed Antibody-Conjugated Beads** (vortex for at least ≥ 15 seconds before addinguse) and 50 µL of sample or standard to each well of a 96-well plate. Cover the plate with a sealing film and incubate on a microplate shaker at room temperature, 600 rpm, protected from light, at 600 rpm for 1 hour.

2. **Detection antibody incubation**

2. After incubation, place the plate on a magnetic separator for 1 minute. Decant and remove the supernatant. , then aAdd 100 µL of **Biotinylated Detection Antibodies** to each well. Ccover the plate, with a sealing film and incubate under same conditions on a microplate shaker at room temperature, protected from light, at 600 rpm for 1 hour.

3. **SA-PE incubation and washing**

3. After incubation, place the plate on a magnetic separator for 1 minute. Decantremove the supernatant, and wash once withadd 200 µL of **Wash Buffer** to each well. place the plate on the magnetic separator for 1 minute, and decant the supernatant. Remove the wash buffer. Then,and add 100 µL of **SA-PE** to each well. Cover the plate with a sealing film and incubate on a microplate shaker at room temperature, 600 RPM, protected from light, at 600 rpm for 30 minutes. 0.5 hours. Following incubation, wash twice with 200 µL of Wash Buffer, separating each time.

4. **Final resuspension and detection**

After incubation, place the plate on a magnetic separator for 1 minute. Decant the supernatant, add 200 µL of **Wash Buffer** to each well, place the plate on the magnetic separator for 1 minute, and decant the supernatant. Finally, add Resuspend the beads in 200 µL of **Wash Buffer** to each well to resuspend the beads.

and Pproceed with detection using the a flow cytometer under appropriate instrumentsettings.

(The eExperimental protocolconditions may be adjusted by the user according to their specific experimental conditions and sample type or available equipment.)

## Flow Cytometry Detection

**Sample Test Data Acquisition**

1) Detect samples using this kit (example for Acquire samples



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on a flow cytometer using manual gating analysis.

2) Create an FSC-H/SSC-H scatter plot. Adjust the gain of FSC and SSC to gateidentify the cytokine bead population using a rectangular P1 gate, as shown in (Figure 1).

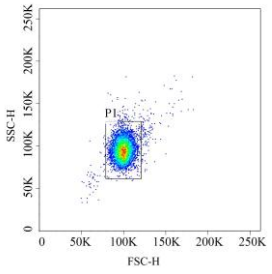


Figure 1

3) Establish Create an APC-H/APC-Cy7-H scatter plot. Identify the individual bead populations fFrom the main P1-gated bead cluster population, identify individual bead populations and gate each specific cytokine-specific bead population using rectangular gates, as shown in (Figure 2).

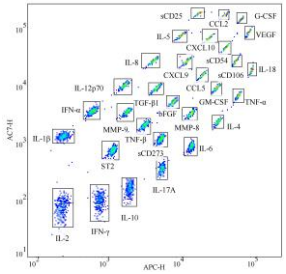


Figure 2

## Data Analysis

1) Open the template for detection and sSet thedata acquisition to collect at least 200 beads per cytokine gate. For example, when analyzing four bead populations, collect if detecting four distinct bead populations, a minimum of 4 × 200 = 800 total beads should be collected. Proceed with data analysis after acquisition is complete.

2) Calculate the median fluorescence intensity (MFI) of the standard and samples replicate wells., then sSubtract the MFI value of the blank well to obtain the corrected MFI value.

2)3) -Generate a standard curve by plotting concentration on the (x-axis) and MFI on the (y-axis) using a log-log scale, and fit the curve the data with a four4-parameter logistic (4-PLK) functionmodel.

3)4) If the sample MFI exceeds the upper limit of the standard curve, appropriately dilute the sample appropriately and repeat the measurement. Multiply the calculated concentration by the corresponding dilution factor.

5) Data Analysis: Analyze Experimental data can be analyzed using the Elabscience® Aptplex™ Analysis Software or other commercially available compatible data analysis software.

4)

## Performance parameters

1. Detection range:

| Cytokines | Range of linearity |
|---|---|
| IL-6 | 5-5000 pg/mL |
| IL-1β | 5-5000 pg/mL |
| TNF-α | 5-5000 pg/mL |
| IFN-γ | 5-5000 pg/mL |
| CXCL10(IP-10) | 10-5000 pg/mL |
| CCL2(MCP-1) | 10-5000 pg/mL |
| IL-8(CXCL8) | 5-5000 pg/mL |
| IL-10 | 5-5000 pg/mL |
| **Cytokines** | **Range of linearity** |
| IL-17A | 5-5000 pg/ml |
| IL-6 | 5-5000 pg/ml |
| TNF-α | 5-5000 pg/ml |
| IFN-γ | 5-5000 pg/ml |
| IL-12p70 | 5-5000 pg/ml |
| IL-10 | 5-5000 pg/ml |
| CCL5（RANTES） | 10-5000 pg/ml |
| CXCL10（IP-10） | 10-5000 pg/ml |
| IL-1β | 5-5000 pg/ml |
| IL-18 | 10-5000 pg/ml |
| sCD54(ICAM-1) | 40-10000 pg/ml |
| IL-2RA(sCD25) | 10-5000 pg/ml |

2. Limit of Blank (LoB):

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2. The LoB for all assays analytes is ≤ 8 pg/mL.

3. Recovery:

3. The mean recovery rate ranges from 70% to 120%.

4. Precision:

4. Both The intra-assay and inter-assay coefficients of variation (CV) are ≤ 15%.

5. Specificity:

5. No significant cross-reactivity is observed among the different factors analytes withinincluded in this kit.

## Precautions

1. This product is intended for **research use only** and must be used by qualified professionals only. Personnel responsible for data interpretation and reporting must receive propershould have appropriate technical training.
2. Please observe all safety precautions and adhere to standard laboratory reagent handling proceduresFollow standard laboratory safety practices and reagent handling procedures. This product contains fluorescent dyes. Avoid direct contact with skin and eyes, and do not allowprevent contamination of food and beverages., and Aalways wear appropriate gloves whenpersonal protective equipment, including gloves, during handling.
3. Incorrect Improper calibration of the flow cytometer calibration, improper inadequate compensation for fluorescence compensation spillover, or imprecise incorrect gating strategies may lead to erroneousinaccurate results. Please rRefer to the instrument's operating manufacturer's manual forand ensure proper calibration procedures to sample acquisition ensure optimal performance prior to use.
4. Prior to Before use, thoroughly vortex the bead suspension thoroughly to ensure homogeneity uniform bead dispersion and to prevent bead aggregation, which could lead to uneven bead distribution across wells may affect assay performance.
5. To prevent cross-contamination,: change pipette tips between each well-, exercise caution when removing the plate sealer to avoid contact with adjacent wells; always use newfresh tips when handlingfor different standards or samples,: and avoid generating bubbles formation during pipetting. The uUse of a multichannel pipette is recommended for adding wash buffersteps.
6. To ensure fluorescence detection quality, protect the reaction from light during all steps involving detection antibodies Protect all reactions involving detection antibodies and SA-PE from light throughout the assay to maintain fluorescence signal integrity.
7. Do not mix reagents from different lot numbers. Do not use products from or substitute reagents from other manufacturers interchangeably. Strictly follow the instructions provided in this manual. Use the product only within its stated validity period Use all components according to the instructions provided in this manual and within their stated expiration dates.

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