

## Aptplex™ Human Cytokine 3-Plex Panel(C)

Catalog No: MPD002

Product size: 96 T

### Intended Use

Aptplex™ Human Cytokine 3-Plex Panel(C) 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:

CCL2(MCP-1), CCL5(RANTES), CXCL10(IP-10), CXCL9(MIG), G-CSF,GM-CSF, IFN- $\alpha$ , IFN- $\gamma$ , IL-10,IL-12p70, IL-17A, IL-18, IL-1 $\beta$ , IL-2, IL-2RA(sCD25), IL-4, IL-5, IL-6, IL-8(CXCL8), LTA, MMP-8, MMP-9, sCD106(VCAM-1), sCD273(sPD-L2), sCD54(ICAM-1), ST2, TGF- $\beta$ 1(Free active), TNF- $\alpha$ , VEGF, bFGF

### Components

Component	Component Name	96T	Storage
MPD002A	Cytokines Capture Beads	4.8mL×1	2-8°C shading light
MPD002B	Biotinylated Antibody	9.6 mL×1	2-8°C
MPD002C	SA-PE	9.6 mL×1	2-8°C shading light
MPD002D	Standard	2vials	2-8°C
MPD002E	Assay Buffer	5mL×1	2-8°C
MPD002F	Wash Buffer	30mL×2	2-8°C
	Plate Sealing Film	5 pieces	
	Manual	1 copy	

### Detection Principle

The Aptplex™ assay is a multiplex bead based immunoassay that uses antibody-conjugated magnetic beads with distinct fluorescence intensities to capture target antigens simultaneously. Each target antigen is recognized by a specific capture antibody on the bead and binds with a corresponding biotinylated detection antibody to form a bead-analyte-detection antibody “sandwich complex”. Streptavidin-phycoerythrin (SA-PE) binds to the biotinylated detection antibodies producing a fluorescent signal proportional to the amount of each analyte. The fluorescence of each bead is measured using flow cytometry and correlated with a standard curve to determine analyte concentrations.

### Detection Sample Types

Serum  EDTA Plasma

### Storage

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

#### 1) Serum

Allow whole blood to clot for 1 hour at room temperature or overnight at 2-8 °C, then centrifuge for 20 min at 1000 × g at 2-8 °C. Collect the supernatant for the assay.

#### 2) Plasma

Collect using EDTA-Na<sub>2</sub> as an anticoagulant. Centrifuge 15 min at 1000 × g at 2-8 °C within 30 min of collection. Collect the supernatant for the assay.

### Materials Not Supplied

- Vortex mixer
- 96-well plate thermostatted shaking incubator
- 96-well plate centrifuge
- Flow cytometer (2-laser 6-color configuration, equipped with PE, APC and APC/Cy7 detection channels)
- Magnetic separation plate or magnetic rack.
- **Centrifuge-based washing:** clear U-bottom 96-well plates, 96-well plate centrifuge
- **Magnetic bead-based washing:** clear flat-bottom 96-well plates, magnetic separation plate or magnetic rack

### Standard Preparation Procedure

1. Prepare eight 0.6 mL microcentrifuge tubes and label them 0-7. Leave tube 7 empty. Add 150  $\mu$ L of **Assay Buffer** to tubes 0-6.

#### 2. Reconstitute the lyophilized standard

Briefly centrifuge the tube at 500 × g for 10 s to collect the powder at the bottom.

Add 500  $\mu$ L of **Assay Buffer** to the vial. Let it stand for 5 min.

Mix gently with a pipettor until the standard is completely dissolved.

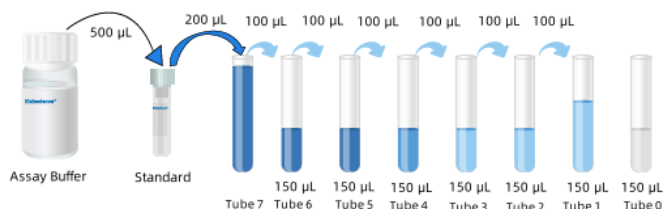
Transfer 200  $\mu$ L solution to tube 7. This is the highest concentration standard.

#### 3. Prepare serial dilutions

- Transfer 100  $\mu$ L from tube 7 into tube 6 and mix thoroughly

(1:2.5 dilution).

- Serially dilute by transferring 100  $\mu$ L from each tube to the next lower tube (tube 6  $\rightarrow$  5  $\rightarrow$  4  $\rightarrow$  3  $\rightarrow$  2  $\rightarrow$  1), mixing thoroughly at each step.
- Tube 0 contains Assay Buffer only and serves as the zero standard.



Note: The concentration of the highest standard solution may vary (refer to the product COA for details).

## Procedure

### 1. Bead and Sample incubation

Add 50  $\mu$ L of **Premixed Antibody-Conjugated Beads** (vortex  $\geq$  15 s before use) and 50  $\mu$ L 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, for 1 hour.

### 2. Detection antibody incubation

After incubation, place the plate on a magnetic separator for 1 minute and remove the supernatant. Add 100  $\mu$ L of **Biotinylated Detection Antibodies** to each well, cover the plate, and incubate under same conditions for 1 hour.

Note: This step can also be accomplished through centrifugation separation steps. Specifically, remove the plate seal. Centrifuge the 96-well plate at 300  $\times$  g for 5 min

and remove the supernatant. Add 100  $\mu$ L of Biotinylated Detection Antibodies to each well, cover the plate, and incubate under same conditions for 1 hour.

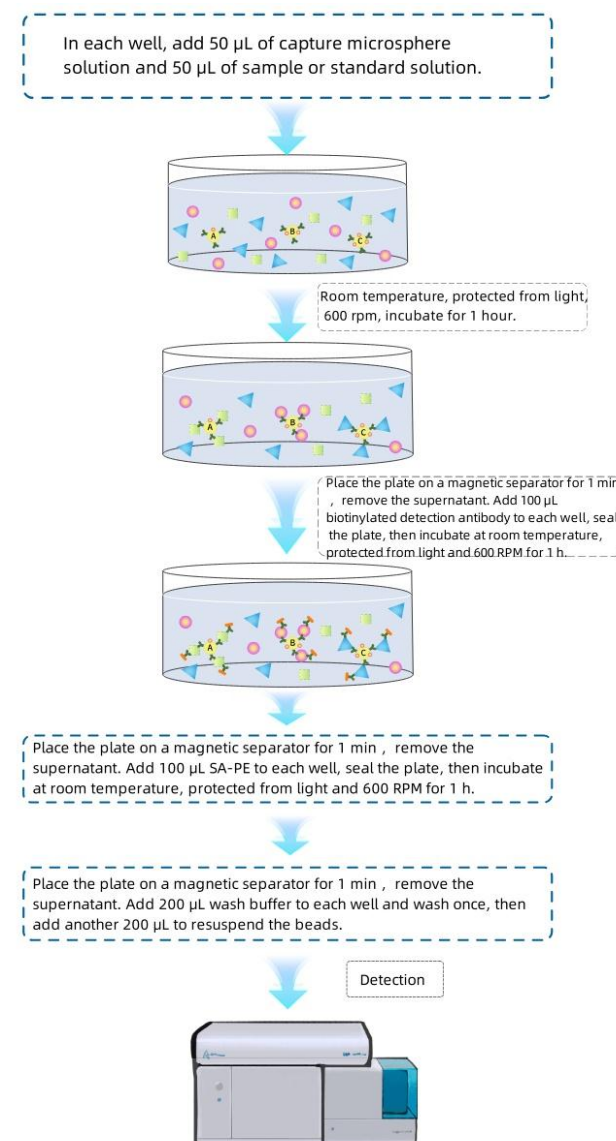
### 3. SA-PE incubation and washing

After incubation, place the plate on a magnetic separator for 1 minute remove the supernatant, and wash once with 200  $\mu$ L of **Wash Buffer**. Remove the wash buffer. and add 100  $\mu$ L of **SA-PE** to each well. Cover the plate with a sealing film and incubate on a shaker at room temperature, 600 RPM, protected from light, for 30 minutes. Following incubation, wash once with 200  $\mu$ L of Wash Buffer, separating each time.

Note: The washing procedure can also be accomplished through centrifugation separation steps. Specifically, remove the plate seal. Centrifuge the 96-well plate at 300  $\times$  g for 5 min. Remove the wash buffer, and add 100  $\mu$  L of SA-PE to each well. Cover the plate with a sealing film and incubate on a shaker at room temperature, 600 RPM, protected from light, for 30 minutes. Following incubation, centrifuge the 96-well plate at 300  $\times$  g for 5 min and discard supernatant.

### 4. Final resuspension and detection

Resuspend the beads in 200  $\mu$ L of **Wash Buffer** and proceed with detection using the a flow cytometer under appropriate settings.



图例:

- Premixed antibody-conjugated bead
- biotinylated detection antibody
- Analytes

