MagPro Human Pulmonary Fibrosis (IPF) 14-Plex

Instruction Manual

Catalog No.: MPES0074 | Product size: 96 T | Version 1.0

Intended Use

The Assay Genie MagPro Human Pulmonary Fibrosis (IPF) 14-Plex 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 the following cytokines in human serum, plasma, cell culture supernatants, and other biological fluids:

TGF-β1, IL-6, TNF-α, IL-1β, IL-18, IL-10, CCL2 (MCP-1), VEGF, MMP-9, sCD54 (ICAM-1), IL-8 (CXCL8), CCL5 (RANTES), IL-17A, and ST2.

Detection Principle

The Assay Genie multiplex assay is a 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
- Cell culture supernatants
- Other biological fluids

Components

Component Name	96T	Storage
Premixed Antibody-Conjugated Beads	2.4 mL × 2	2–8 °C, protected from light
Biotinylated Detection Antibodies	4.8 mL × 2	2–8 °C
SA-PE (ready to use)	4.8 mL × 2	2–8 °C, protected from light
Lyophilized Standard	2 vials	2–8 °C
Assay Buffer	5 mL × 1	2–8 °C
Wash Buffer	30 mL × 2	2–8 °C
Plate Sealing Film	5 pieces	—
Manual	1 copy	—

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

Materials Not Supplied

- U-bottom 96-well transparent plates
- Vortex mixer
- Incubator suitable for 96-well plate
- Magnetic separator
- Flow cytometer (with PE, APC, and APC/Cy7 detection channels)

Sample Collection and Preparation

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_2 as an anticoagulant. Centrifuge 15 min at 1000 × g at 2–8 °C within 30 min of collection. Collect the supernatant for the assay.

3) Cell Culture Supernatant or Other Biological Fluids

Centrifuge 20 min at 1000 × g at 2–8 °C. Collect the supernatant for the assay.

Standard Preparation Procedure

1. Prepare eight 0.6 mL microcentrifuge 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:**
   - Briefly centrifuge the tube at 500 × g for 10 s to collect the powder at the bottom.
   - Add 500 µL of Assay Buffer to the vial. Let it stand for 5 min.
   - Mix gently until the lyophilized standard is completely dissolved.
   - Transfer the entire solution to tube 7. This is the highest concentration standard.
3. **Prepare serial dilutions:**
   - Transfer 50 µL from tube 7 into tube 6 and mix thoroughly (1:3.2 dilution).
   - Serially dilute by transferring 100 µL from each tube to the next lower tube (tube 6 → 5 → 4 → 3 → 2 → 1), mixing thoroughly at each step.
   - Tube 0 contains Assay Buffer only and serves as the zero standard.

Note: The exact concentration of the highest standard is indicated on the standard vial label.

Assay Procedure

1. Bead and Sample Incubation

Add 50 µL of Premixed Antibody-Conjugated Beads (vortex ≥ 15 s before use) 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, 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 µL of Biotinylated Detection Antibodies to each well, cover the plate, and incubate under the 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 µL of Wash Buffer. Remove the wash buffer and add 100 µL 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 twice with 200 µL of Wash Buffer, separating each time.

4. Final Resuspension and Detection

Resuspend the beads in 200 µL of Wash Buffer and proceed with detection using a flow cytometer under appropriate settings.

Experimental conditions may be adjusted according to sample type or available equipment.

Flow Cytometry Detection

Sample Test Data Acquisition

1) Acquire samples on a flow cytometer using manual gating analysis.
2) Create an FSC-H / SSC-H scatter plot. Adjust FSC and SSC to identify the cytokine bead population using a rectangular P1 gate (Figure 1).

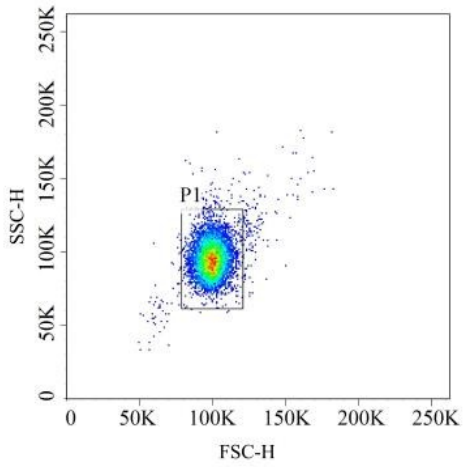

Figure 1. FSC-H vs SSC-H scatter plot with rectangular P1 gate identifying the cytokine bead population.

3) Create an APC-H / APC-Cy7-H scatter plot. From the P1-gated bead population, identify individual bead populations and gate each cytokine-specific bead population using rectangular gates (Figure 2).

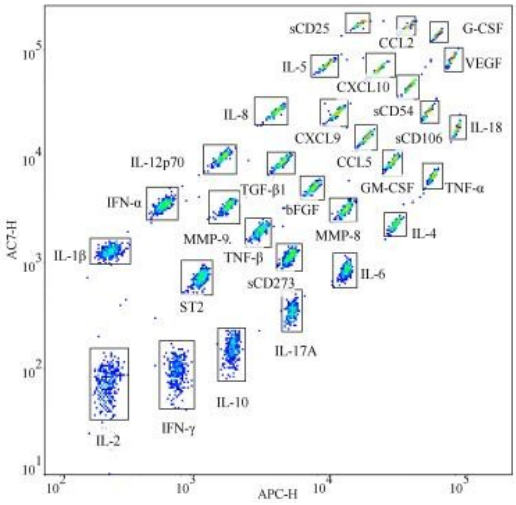

Figure 2. APC-H vs APC-Cy7-H scatter plot showing individual bead populations, each gated to a specific analyte.

Data Analysis

1) Set data acquisition to collect at least 200 beads per cytokine gate. For example, when analyzing 14 bead populations, collect a minimum of 2800 total beads.
2) Calculate the median fluorescence intensity (MFI) of standard and samples. Subtract the MFI of the blank well to obtain the corrected MFI value.
3) Generate a standard curve by plotting concentration (x-axis) and MFI (y-axis) using a log-log scale, and fit the curve with a four-parameter logistic (4-PL) model.
4) Calculate the concentration of unknown samples from the calibration curve of each analyte.
5) If the sample MFI exceeds the upper limit of the standard curve, dilute the sample appropriately and repeat the measurement. Multiply the calculated concentration by the corresponding dilution factor.

Performance Parameters

1. Detection Range

Analyte	Range of Linearity
TGF-β1	10-5000 pg/mL
IL-6	5-5000 pg/mL
TNF-α	5-5000 pg/mL
IL-1β	5-5000 pg/mL
IL-18	10-5000 pg/mL

Analyte	Range of Linearity
IL-10	5-5000 pg/mL
CCL2 (MCP-1)	10-5000 pg/mL
VEGF	10-5000 pg/mL
MMP-9	10-5000 pg/mL
sCD54 (ICAM-1)	40-10000 pg/mL
IL-8 (CXCL8)	5-5000 pg/mL
CCL5 (RANTES)	10-5000 pg/mL
IL-17A	5-5000 pg/mL
ST2	20-10000 pg/mL

2. Limit of Blank (LoB)

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

3. Recovery

The mean recovery ranges from 70 % to 120 %.

4. Precision

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

5. Specificity

No significant cross-reactivity is observed among the analytes included in this kit.

Precautions

1. This product is intended for research use only and must be used by qualified professionals. Personnel responsible for data interpretation and reporting should have appropriate technical training.
2. Follow standard laboratory safety practices and reagent handling procedures. This product contains fluorescent dyes. Avoid direct contact with skin and eyes, prevent contamination of food and beverages, and always wear appropriate personal protective equipment, including gloves, during handling.
3. Improper flow cytometer calibration, inadequate fluorescence compensation, or incorrect gating strategies may lead to inaccurate results. Refer to the instrument manufacturer’s manual and ensure proper calibration prior to sample acquisition.

4. Before use, vortex the bead suspension thoroughly to ensure uniform bead dispersion and to prevent bead aggregation, which 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, use fresh tips for different standards or samples, and avoid bubble formation during pipetting. Use of a multichannel pipette is recommended for wash steps.
6. 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 or substitute reagents from other manufacturers. Use all components according to the instructions provided in this manual and within their stated expiration dates.