Human Cytokine 4-Plex Assay Kit

(Flow Cytometry Multiplex Bead Assay)

Catalog No.: RK05219A

For the simultaneous quantitative determination of multiple human cytokine concentrations in cell culture supernatants, serum, and plasma.

Version: 1.0

*This package insert must be read in its entirety before using this product.

For Research Use Only. Not for use in diagnostic procedures

Introduction

This kit is intended for the detection of IL-4, IL-6, IL-10, and IFN-γ expression in human biological specimens or cell culture supernatants.

Principle of the Assay

This assay is a multiplex sandwich immunoassay based on flow cytometry.

Capture antibodies specific to IL-4, IL-6, IL-10, and IFN-γ are covalently coupled to fluorescently encoded microspheres.

The microspheres are incubated with samples or standards, allowing target analytes to bind to the capture antibodies. After washing, biotinylated detection antibodies are added to form sandwich complexes. Streptavidin-phycoerythrin (SA-PE) is then introduced, binding to the biotin on the detection antibodies.

The microspheres are analyzed using a flow cytometer equipped with dual lasers.

PE fluorescence intensity is directly proportional to the amount of bound analyte, while the microsphere's internal fluorescence identifies the analyte type.

Analyte concentrations are determined by interpolation from a standard curve.

Material Provided & Storage Conditions

Store the unopened kit at 2-8℃. Do not use past the kit expiration date.

Part	Description	Specifications and Quantity
		96 test
Capture Beads	Coupled human IL-4, IL-6, IL-10, and IFN-γ antibody with magnetic beads.	2 mL，1 vial
Standard Lyophilized	Lyophilized antigens of IL-4, IL-6, IL-10, and IFN-γ.	2 vials
Detection Antibodies	Biotinylated antibody cocktail against IL-4, IL-6, IL-10, and IFN-γ.	5 mL，1 vial
Fluorophore Solution	Streptavidin-Phycoerythrin (SA-PE)	5 mL，1 vial
Standard/Sample Diluent	Phosphate-buffered diluent	20 mL，1 vial
Wash Buffer (10×)	10 × concentrated PBST buffer	20 mL，1 vial

Other Supplies Required

1. Single-channel pipette, multi-channel pipette, and pipette tips
2. Magnetic plate (compatible with 96-well microplates) or magnetic stand (compatible with 1.5 mL microcentrifuge tubes)
3. Polypropylene microcentrifuge tubes for sample collection or dilution
4. Ultrapure water (deionized or distilled)
5. Plate seals (sealing film)
6. Reaction plate (microplate)
7. Constant temperature incubator
8. Flow cytometer equipped with two lasers:

- 488 nm or 532 nm excitation, 633 nm emission detection
- With corresponding detection channels for PE, APC, and APC-Cy7.

Precautions

1. Do not use this product after the expiration date stated on the label.
2. Do not mix reagents from different batches or different manufacturers.
3. If the measured sample concentration exceeds the highest standard, dilute the sample with the provided sample diluent.
4. Assay performance is affected by factors including pipetting, washing, incubation time, and temperature. Strict adherence to the protocol and proper record-keeping are required.
5. Sample collection, processing, and storage conditions may affect test results. Follow established procedures carefully.
6. Interference from unknown sample components cannot be completely excluded.
7. There may be harmful substances in the related reagents. Please wear gloves during use and, if necessary, wear goggles.
8. Store reagent components as indicated on the label or in the instruction manual to ensure optimal performance.
9. Adequate mixing of prepared reagents is essential for optimal assay performance. However, some proteins and antibodies are sensitive to vigorous vortexing, which may cause denaturation or loss of activity. Use high-speed vortex mixing with caution.
10. Use sterile consumables when preparing reagents to avoid contamination that may compromise test results.
11. Do not freeze or reuse dissolved standard protein or working solutions.
12. Protect the product from light during storage and use.
13. Use a fresh pipette tip when dispensing different samples to avoid cross-contamination.

14. Prepare reagents strictly according to the instructions. If the kit is used in multiple sessions, prepare only the required amount of working reagents and use immediately. All kit components should be used within one month after opening.

15. Data interpretation note:

This flow cytometry-based multiplex assay employs different detection principles, signal acquisition, and calibration systems compared to ELISA or other immunoassays. Therefore, absolute values obtained from different methods may not be directly comparable. When cross-method data comparison or integration is intended, it is recommended to perform correlation analysis to evaluate consistency and trends between platforms.

Sample Collection & Storage

The sample collection and storage conditions listed below are intended as general guidelines.

Cell Culture Supernatant: Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20°C Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or Heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles. (Note: Citrate plasma has not been validated for use in this assay.)

Note**:** Avoid hemolyzed and lipemic samples for serum and plasma.

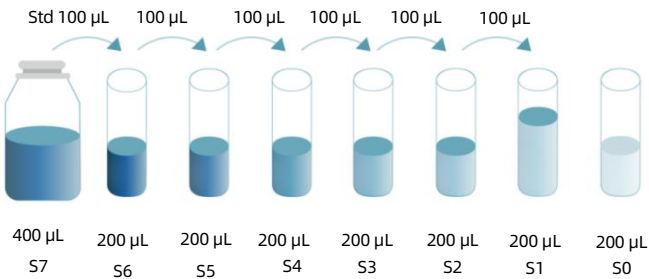
Reagent Preparation

Bring all reagents to room temperature before use. If crystals have formed, warm the reagent to room temperature and mix gently until completely dissolved.

Standard-Add 400 µL of sample diluent to the vial containing the freeze-dried calibrator (S7). Allow to stand for 15 minutes, then vortex gently for 5 seconds. Repeat this cycle three times.

Label seven microcentrifuge tubes as S6 through S0. Add 200 µL of sample diluent to each tube.

Transfer 100 µL from S7 to S6 and mix gently by vortexing for 5 seconds. Then transfer 100 µL from S6 to S5. Continue this serial dilution through S1. S0 serves as the zero calibrator.



Wash Buffer-Dilute the wash buffer concentrate 1:10 with double-distilled or deionized water. Mix 1 part concentrate with 9 parts water.

Sample Preparation

Note: To protect the kit reagents from contamination, wear a face mask and gloves during the entire procedure.
Cell culture supernate samples require a appropriate dilution with Sample Diluent.
Serum (pretreated) and plasma samples require a appropriate dilution with Sample Diluent.

Assay Procedure

To prevent bacterial growth, immediately store any unused reagents at 2-8℃ after each use.

1. Reagent Preparation: Prepare the kit and dilute the 10× wash buffer 1:10 with deionized water.

2. Plate Layout: Define the plate layout and designate wells for standards and samples.

3. Microsphere Incubation: Vortex the microsphere suspension thoroughly. Add 20 μL/well to each well. Add 50 μL/well of standard or sample according to the plate layout. Seal the plate. Mix thoroughly and incubate at 37°C, 1,200 rpm, protected from light for 30 minutes.Place the plate on a magnetic plate and allow magnetic separation for 2 minutes.Remove the seal and decant the supernatant. Blot excess liquid with absorbent paper.

4. Washing: Add 100 μL of 1× wash buffer to each well. Shake on a constant temperature incubator at 1,200 rpm for 1 – 2 minutes. Place on the magnetic plate and magnetize for 2 minutes. Decant the supernatant and absorb residual liquid with absorbent paper.

5. Detection Antibody Addition: Add 50 μL of detection antibody solution to each well. Seal with a plate sealer. Incubate at 37°C with shaking at 1,200 rpm for 20 minutes protected from light. Remove the plate, place on the magnetic plate, magnetize for 2 minutes. Decant the supernatant and absorb residual liquid with absorbent paper.

6. SA-PE Incubation: Add 50 μL of fluorophore solution to each well. Seal with a plate sealer. Incubate at 37°C with shaking at 1,200 rpm for 10 minutes protected from light. Remove the plate, place on the magnetic plate, magnetize for 2 minutes. Decant the supernatant and absorb residual liquid with absorbent paper.

7. Washing: Repeat Step 4 once.

8. Reading: Remove the reaction plate from the magnetic plate, add 200 μL of 1x washing solution to each well, mix well, and select an appropriate container for machine detection.

Assay Procedure Summary

Prepare the samples and reagents that need to be tested

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Add 20 μL of the microsphere suspension to each well

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Add 50 μL of the calibrator or the sample to be tested to each well. Incubate at 37°C for 30 minutes, then wash once

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Add 50 μL of the detection antibody solution, incubate at 37°C for 20 minutes, and then remove the supernatant by magnetic separation

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Add 50 μL of fluorescein solution, incubate at 37°C in the dark for 10 minutes, and then wash once

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Add 200 μL of the 1x washing solution

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Using flow cytometry for detection

Flow Cytometry Parameter Setup and Data Acquisition

1. Parameter Setup

Before acquiring samples, resuspend the microsphere suspension thoroughly and dilute 20 µL of the stock with 180 µL of wash buffer. Load the diluted microspheres onto the flow cytometer.

Adjust FSC and SSC parameters to identify the microsphere population and draw a gate around it.

Use the APC and APC-Cy7/APC-A750 channels to resolve the microsphere subsets based on their distinct fluorescence intensities.

Adjust the PE channel voltage so that all microsphere clusters fall within the scale and do not saturate the detector.

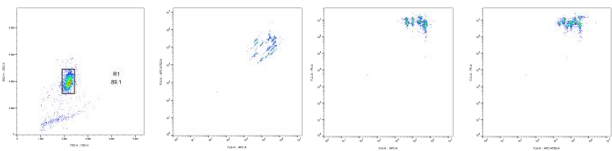


Figure 1: Create a scatter plot with FSC on the X-axis and SSC on the Y-axis. Gate the microsphere population (R1).

Figure 2: Create a scatter plot with APC on the X-axis and APC-A750 on the Y-axis. Gate individual cytokine populations from the microsphere gate.

Figures 3 and 4: Create scatter plots with APC vs. PE and APC-A750 vs. PE, respectively. Display the gated microspheres to ensure all microsphere clusters are clearly and distinctly distributed on the plots, showing the PE fluorescence intensity for each cytokine.

2. Data Collection

Acquire each standard and sample on the flow cytometer. Collect at least 100 - 200 events per microsphere subset, corresponding to a minimum of 400 - 800 total events for this 4-plex kit.

3. Data Analysis

Acquired data can be analyzed automatically using FCAP Software. If FCAP software is not available, manual analysis may be performed as follows:

Gate the total microsphere population using FSC vs. SSC. Within this gate, 4 distinct microsphere subsets—corresponding to the 4 cytokine detection beads—are resolved in the APC and APC-Cy7 channels.

For each standard and sample, record the mean fluorescence intensity (MFI) of each microsphere subset in the PE channel. Export the MFI values and corresponding standard concentrations to Excel. Perform log-log transformation (log of concentration vs. log of MFI) to generate a standard curve.

Test Results Interpretation

This product quantifies IL-4, IL-6, IL-10, and IFN-γ by interpolating against a standard curve generated from calibrators.

If a result exceeds the upper limit of quantification (ULOQ), dilute the sample with Sample Diluent and re-assay.
If a result is below the lower limit of quantification (LLOQ), report as <LLOQ.

Results reflect analyte concentrations at the time of sample collection. Diagnostic conclusions should be made in conjunction with clinical findings.

Sensitivity

Analyte	Sensitivity	Analyte	Sensitivity
IL-4	≤2.5 pg/mL	IL-10	≤2.5 pg/mL
IL-6	≤2.5 pg/mL	IFN-γ	≤2.5 pg/mL

Recovery

Analyte	Recovery	Analyte	Recovery
IL-4	70 - 130%	IL-10	70 - 130%
IL-6	70 - 130%	IFN-γ	70 - 130%

Linearity

Analyte	linearity	Analyte	linearity
IL-4	6.68-5000 pg/mL	IL-10	6.68-5000 pg/mL
IL-6	6.68-5000 pg/mL	IFN-γ	6.68-5000 pg/mL

Intra-Assay Precision

The coefficient of variation (CV) should be <10%.

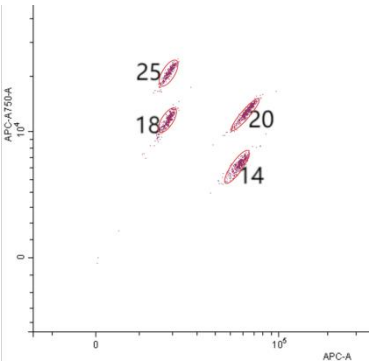
Inter-Assay Precision

The coefficient of variation (CV) should be <15%.

Standard

Analyte	Bead ID	Standard	Analyte	Bead ID	Standard
IL-4	14	5000 pg/mL	IL-10	20	5000 pg/mL
IL-6	18	5000 pg/mL	IFN-γ	25	5000 pg/mL

Microsphere Distribution Map



Precautions

(1) This reagent is only used for in vitro testing.
(2) Before using the reagent kit, it is necessary to check the packaging of each reagent for any liquid leakage.
(3) Users need to undergo necessary training, carefully read the instructions before use, and strictly follow the instructions for operation.
(4) During operation, personal safety protection must be taken to prevent samples, reagents, calibrators, etc. from coming into contact with the human body.
(5) Clinical samples, calibration materials, experimental waste, and other materials should be treated as potential sources of infection and must comply with relevant regulatory requirements.
(6) To ensure accurate and reliable test results, please calibrate regularly. Calibration is required when changing batches of reagents.
(7) Do not mix leftover reagents with newly opened reagents to prevent contamination of the newly opened reagents. Please discard the contaminated reagents.
(8) Different batches of reagents must not be mixed and should be used within their expiration date.
(9) Use absorbent paper to remove residual liquid and avoid contamination between pores.
(10) After the reaction is complete, the sample should be tested on the machine within 4 hours. During the testing process, be careful to avoid light for the sample to be tested.

Information

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Date of instruction manual modification

October 31, 2025