ABplex Human Cytokine 12-Plex Assay Kit

Catalog No.: RK04296A

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-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IFN-α, IFN-γ, and TNF-α 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-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IFN-α, IFN-γ, and TNF-α 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 an ABplex-100 Multiplex Flow Cytometer.

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 °C. Do not use past the kit expiration date.

Part	Description	Specifications and Quantity
		96 tests
Capture Beads	Coupled human IL-1 β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IFN-α, IFN-γ, and TNF-α antibody with magnetic beads.	2 mL,1 vial
Standard Lyophilized	Lyophilized antigens of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IFN-α, IFN-γ, and TNF-α.	2 vials
Detection Antibodies	Biotinylated antibody cocktail against IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IFN-α, IFN-γ, and TNF-α.	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. Vortex Mixer
9. ABplex-100 Multiplex Flow Cytometer

Precautions

1. For Research Use Only. Not for use in diagnostic procedures.
2. This product is for in vitro use only.
3. Wear appropriate personal protective equipment (gloves, safety goggles, and lab coat) when handling reagents. Some reagents contain hazardous substances.
4. Treat all clinical samples, calibrators, and experimental waste as potentially infectious materials in accordance with local regulations.
5. Do not use the product after the expiration date stated on the label.
6. Do not mix reagents from different batches or manufacturers.
7. Store reagent components as indicated on the label to ensure optimal performance.
8. Protect the product from light during storage and use.
9. Do not freeze or reuse reconstituted standard protein or working solutions.
10. Use sterile consumables when preparing reagents to avoid contamination.
11. 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.
12. Strict adherence to the protocol is required. Assay performance is affected by pipetting technique, washing efficiency, incubation time, and temperature.
13. Sample collection, processing, and storage conditions may affect test results. Avoid hemolyzed or lipemic samples.
14. Adequate mixing of prepared reagents is essential. However,

avoid vigorous vortexing of proteins and antibodies to prevent denaturation.

15. Use a fresh pipette tip when dispensing different samples to prevent cross-contamination.

16. Regular calibration is required to ensure accurate and reliable test results.

17. Check the packaging of each reagent for any liquid leakage before use.

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 1,000 × 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 1,000 × 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.

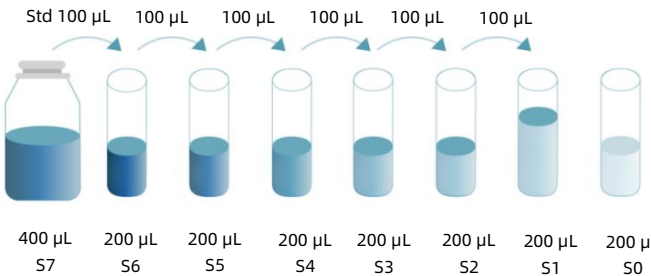
Reagent Preparation

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 supernatant samples require an appropriate dilution with Sample Diluent.
Serum and plasma samples require an appropriate dilution with Sample Diluent.

Assay Procedure

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

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

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

4. Microsphere Incubation: Vortex the microsphere suspension thoroughly. Add 20 µL 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 ℃, 1,200 rpm, protected from light for 30 minutes.Place the plate on a magnetic plate for 2 minutes to allow magnetic separation.Remove the seal and decant the supernatant. Blot excess liquid with absorbent paper.

5. 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.

6. 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.

7. 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.

8. Washing: Repeat Step 4 once.

9. Reading: Remove the reaction plate from the magnetic plate, add 70 µL of 1× wash buffer 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 fluorophore solution, incubate at 37 °C in the dark for 10 minutes, and then wash once

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Add 70 μL of the 1× wash buffer

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Detected using the ABplex-100 instrument

Data Acquisition

1. Standard Curve Generation
Plot the standard curve using the mean fluorescence intensity (MFI) values obtained for the standards versus their respective concentrations. A five-parameter logistic (5-PL) curve fit is recommended for optimal accuracy. Alternative curve fitting methods (e.g., four-parameter logistic, log-log) may be applied as appropriate for the specific assay requirements.

2. Concentration Determination
The concentration of each sample is determined by interpolating its observed MFI value from the generated standard curve. It is essential that sample MFI values fall within the dynamic range of the standard curve for accurate quantification.

3. Dilution Factor Correction
If samples were diluted prior to analysis, multiply the concentration obtained from the standard curve by the applicable dilution factor to calculate the final concentration in the original, undiluted sample.

Test Results Interpretation

This product quantifies IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IFN-α, IFN-γ, and TNF-α 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.
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.

LIMITATIONS

Interference from unknown sample components cannot be completely excluded.
If the measured sample concentration exceeds the highest standard, dilute the sample with the provided Sample Diluent and re-assay.
Diagnostic conclusions should be made in conjunction with clinical findings.

Sensitivity

Analyte	Sensitivity	Analyte	Sensitivity
IL-1β	≤2.5 pg/mL	IL-10	≤2.5 pg/mL
IL-2	≤2.5 pg/mL	IL-12p70	≤2.5 pg/mL
IL-4	≤2.5 pg/mL	IL-17A	≤2.5 pg/mL
IL-5	≤2.5 pg/mL	IFN-γ	≤2.5 pg/mL
IL-6	≤2.5 pg/mL	IFN-α	≤2.5 pg/mL
IL-8	≤2.5 pg/mL	TNF-α	≤2.5 pg/mL

Recovery

Analyte	Recovery	Analyte	Recovery
IL-1β	70 - 130%	IL-10	70 - 130%
IL-2	70 - 130%	IL-12p70	70 - 130%
IL-4	70 - 130%	IL-17A	70 - 130%
IL-5	70 - 130%	IFN-γ	70 - 130%
IL-6	70 - 130%	IFN-α	70 - 130%
IL-8	70 - 130%	TNF-α	70 - 130%

Linearity

Analyte	linearity	Analyte	linearity
IL-1β	6.68-5000 pg/mL	IL-10	6.68-5000 pg/mL
IL-2	6.68-5000 pg/mL	IL-12p70	6.68-5000 pg/mL
IL-4	6.68-5000 pg/mL	IL-17A	6.68-5000 pg/mL
IL-5	6.68-5000 pg/mL	IFN-γ	6.68-5000 pg/mL
IL-6	6.68-5000 pg/mL	IFN-α	6.68-5000 pg/mL
IL-8	6.68-5000 pg/mL	TNF-α	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-1β	44	5000 pg/mL	IL-10	20	5000 pg/mL
IL-2	13	5000 pg/mL	IL-12p70	26	5000 pg/mL
IL-4	14	5000 pg/mL	IL-17A	34	5000 pg/mL
IL-5	12	5000 pg/mL	IFN-γ	25	5000 pg/mL
IL-6	18	5000 pg/mL	IFN-α	27	5000 pg/mL
IL-8	19	5000 pg/mL	TNF-α	21	5000 pg/mL

Microsphere Distribution Map

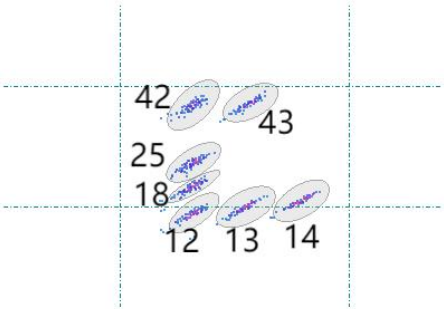


Figure 1. Spectral distribution of capture beads for 12-plex assay.

Information

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