# ABplex Human Cytokine 12-Plex Assay Kit

Catalog NO.:RK04296

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

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

For research use only. Not for use in diagnostic procedures

## Introduction

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of IL-1β、IL-2、IL-4、IL-5、IL-6、IL-8、IL-10、IL-12p70、IL-17A、IFN-α、IFN-γ、TNF-α in human serum, plasma, cell culture supernatants.

## Principle of the Assay

Analyte-specific antibodies are pre-coated onto magnetic beads with fluorophores at set ratios for each unique microparticle region. Coupled beads, standards and samples are pipetted into wells and the immobilized antibodies bind the analytes of interest. After washing away any unbound substances, a biotinylated antibody cocktail specific to the analytes of interest is added to each well. Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (SA-PE), which binds to the biotinylated antibody, is added to each well. Final washes remove unbound Streptavidin-PE, the beads are resuspended in buffer and read using the ABplex-100 Analyzer. A Coupled bead in the analyzer captures and holds the superparamagnetic microparticles in a monolayer. Two spectrally distinct Light Emitting Diodes (LEDs) illuminate the microparticles. One LED excites the dyes inside each microparticle to identify the region and the second LED excites the PE to measure the amount of analyte bound to the microparticle, PE serves as a fluorescent indicator, or reporter. A sample from each well is imaged with a CCD camera with a set of filters to differentiate excitation levels.

## Material Provided & Storage Conditions

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

| Part | Description | Specifications and Quantity | | |
|---|---|---|---|---|
| | | 50 test | 100 test | 200 test |
| beads | Coupled human IL-1β、IL-2、IL-4、IL-5、IL-6、IL-8、IL-10、IL-12p70、IL-17A、IFN-α、IFN-γ、TNF-α antibody with magnetic beads. | 0.25mL, 1 vial | 0.5mL, 1 vial | 1mL, 1 vial |
| Standard Lyophilized | recombinant human IL-1β、IL-2、IL-4、IL-5、IL-6、IL-8、IL-10、IL-12p70、IL-17A、IFN-α、IFN-γ、TNF-α in a buffered protein base with preservatives; | 1 vial | 2 vials | 4 vials |
| Detection Antibodies | Biotin conjugated IL-1β、IL-2、IL-4、IL-5、IL-6、IL-8、IL-10、IL-12p70、IL-17A、IFN-α、IFN-γ、TNF-α antibodies | 2.5mL, 1 vial | 5mL, 1 vial | 10mL, 1 vial |
| Streptavidin-Phycoerythrin | concentrated streptavidin-phycoerythrin conjugate with preservatives. | 2.5mL, 1 vial | 5mL, 1 vial | 10mL, 1 vial |
| Standard/Sample Diluent | a buffered protein base with preservative. | 20mL, 1 vial | 20mL, 1 vial | 20mL, 1 vial |
| Wash Buffer(20x) | a buffered protein base with preservative. | 20mL, 1 vial | 20mL, 1 vial | 20mL, 1 vial |

## Other Supplies Required

1. Adjustable micropipette and pipette tip
2. Constant temperature incubator
3. Magnetic plate

## Precautions

1. Any variation in diluent, operator,pipetting technique,washing technique, incubation time or temperature, and kit age can cause variation in binding.

2. Variations in sample collection, processing, and storage may cause sample value differences.

3. Reagents may be harmful, if ingested,rinse it with an excess amount of tap water.

4. Do not mix or substitute reagents with those from other lots or other sources.

5. Adequate mixing is particularly important for good result. Use a mini-vortexer at the lowest frequency.

6. Mix the sample and all components in the kits adequately, and use clean plastic container to prepare all diluents.

7. Both the sample and standard should be assayed in duplicate, and reagents should be added in sequence in accordance with the requirement of the specification.

8. Reuse of dissolved standard is not recommended.

9. The kit should not be used beyond the expiration date on the kit label.

10. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.

## Sample Collection & Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

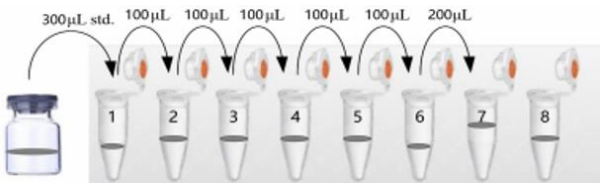
Plasma: Collect plasma using EDTA or Heparin as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles. (Note: Citrate plasma has not been validated for use in this assay.)

**Note**:Avoid hemolytic and hyperlipidemia sample for serum and plasma.

## Reagent Preparation

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

**Standard-**Prepare 8 EP tubes labeled as 1-8 and arrange them in order for diluting calibration standards. Add 300 μ L of reconstitution calibration standard to tube 1, 300 μ L of sample diluent to each of tubes 2-6, and 200uL of sample diluent to tube 7. Transfer 100 μ L from tube 1 to tube 2 and use a pipette to blow and mix thoroughly 10 times. Transfer 100 μ L from tube 2 to tube 3, then transfer to tube 4 and continue to dilute continuously to tube 6. Transfer 200uL from tube 6 to tube 7 for dilution and mix thoroughly. Add 300uL of sample diluent to tube 8 as a zero calibration standard.




**Wash Buffer-**If crystals have formed in the concentrate,warm to room temperature and mix gently until the crystals have completely dissolved.Dilute 1:20 with double distilled or deionized water before use.

## Sample Preparation

**Note** During the whole procedure, it is recommended that a face mask and gloves be used to protect kit reagents from contamination.

**Sample** Different types of sample need appropriate dilutions.

**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

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1.Prepare all reagents,working Standard Lyophilized,and samples as directed in the previous sections.
2.Add 50µl of samples to wells.
3.Resuspend the Coupled beads by vortexing. Add 5µl of the beads to each well of the microplate.Securely cover with a foil plate sealer.Incubate for 1 hours at 37℃on a horizontal orbital microplate shaker set at 1200±50 rpm.
4.Using a magnetic plate to accommodate a microplate, wash by applying the magnet to the bottom of the microplate, allow 2 min before removing the liquid,filling each well with wash buffer (100 µL) and allow 2 min before removing the liquid again.Uniform removal of liquid is essential for good performance.

5.Add 50μL of detection Antibodies to the wells.Securely cover with a foil plate. sealer and incubate for 30 min at 37℃ on the shaker set at 1200±50 rpm.
6.Repeat the wash buffer as in step 4.
7.Add 50μL of Streptavidin-Phycoerythrin to the wells.Securely cover with a foil plate sealer and incubate for 15 minutes at 37℃ on the shaker set at 1200±50 rpm
8. Repeat the wash buffer as in step 6.
9.Resuspend the microparticles by adding 70 μL of wash buffer to the wells. Incubate for 2 minutes on the shaker set at 1200±50 rpm.
10.Using the ABplex-100 analyzer to read.

Note: Resuspend microparticles immediately prior to reading by shaking the plate for 2 minutes on the plate shaker set at 1200±50 rpm.

## Assay Procedure Summary

Prepare the Standard Lyophilized and reagents

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Add 50μL of samples（Standard/Sample diluent 25μL/well+sample 25μL/well）or 50μl Standards to wells

↓

Add 5μL beads to wells

Incubate for 1 hour at 37℃, then wash once

↓

Add 50μL detection antibodies

Incubate for 0.5 hour at 37℃, then wash once

↓

Add 50μL Streptavidin-Phycoerythrin

Incubate for 15 min at 37℃ under dark condition

↓

Add 70μL wash buffer

↓

Detect with the ABplex-100

## Calculation of Results

1.Use the Standard concentrations on the Standard Value Card and calculate 3-fold dilutions for the remaining levels. Average the duplicate readings for each standard and sample and subtract the average blank Median Fluorescence Intensity (MFI).

2.Create a standard curve for each analyte by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit.

Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## Sensitivity

LOD-The concentration of analyte for calculating two concentration of the mean the fluorescence intensity signal of twenty zero standard replicates and three next standard.

| Analyte | Sensitivity (LOD) |
|---|---|
| IL-1β | 3.5 pg/ml |
| IL-2 | 2.5 pg/ml |
| IL-4 | 2.5 pg/ml |
| IL-5 | 2.5 pg/ml |
| IL-6 | 2.5 pg/ml |
| IL-8 | 5 pg/ml |
| IL-10 | 3.5 pg/ml |
| IL-12p70 | 2.5 pg/ml |
| IL-17A | 5 pg/ml |
| IFN- α | 3.5 pg/ml |
| IFN-γ | 3.5 pg/ml |
| TNF-α | 2.5 pg/ml |

## Specificity

The test results are not affected by jaundice (bilirubin<25mg/dL), hemolysis (hemoglobin<28mg/dL), or hyperlipidemia (triglycerides<1500mg/dL).

This assay recognizes both recombinant and natural human Cathepsin B. The factors listed below were prepared at and assayed for cross-reactivity. No significant cross-reactivity was observed with the following:

| | |
|---|---|
| IL-7 | 10000pg/mL |
| IL-13 | 10000pg/mL |

| | |
|---|---|
| IL-15 | 10000pg/mL |
| IL-18 | 10000pg/mL |
| IL-22 | 10000pg/mL |

## Precision

**Inter-Assay Precision** The coefficient of variation (CV) should < 15%。

## Recovery

Adding known concentrations of IL-1β、IL-2、IL-4、IL-5、IL-6、IL-8、IL-10、IL-12p70、IL-17A、IFN-α、IFN-γ、TNF-α to the serum matrix resulted in recovery rates of [85%, 115%].

## Linearity

| Analyte | linear range | \|r\| |
|---|---|---|
| IL-1β | 3.5-5000pg/mL | ≥0.980 |
| IL-2 | 2.5-5000pg/mL | ≥0.980 |
| IL-4 | 2.5-5000pg/mL | ≥0.980 |
| IL-5 | 2.5-5000pg/mL | ≥0.980 |
| IL-6 | 2.5-5000pg/mL | ≥0.980 |
| IL-8 | 5-5000pg/mL | ≥0.980 |
| IL-10 | 3.5-5000pg/mL | ≥0.980 |

| | | |
|---|---|---|
| IL-12p70 | 2.5-5000pg/mL | ≥0.980 |
| IL-17A | 5-5000pg/mL | ≥0.980 |
| IFN- α | 3.5-5000pg/mL | ≥0.980 |
| IFN-γ | 3.5-5000pg/mL | ≥0.980 |
| TNF-α | 2.5-5000pg/mL | ≥0.980 |

## HOOK

| Analyte | HOOK |
|---|---|
| IL-1β | 40000pg/mL |
| IL-2 | 40000pg/mL |
| IL-4 | 40000pg/mL |
| IL-5 | 40000pg/mL |
| IL-6 | 40000pg/mL |
| IL-8 | 40000pg/mL |
| IL-10 | 40000pg/mL |
| IL-12p70 | 40000pg/mL |
| IL-17A | 40000pg/mL |
| IFN- α | 40000pg/mL |
| IFN-γ | 40000pg/mL |
| TNF-α | 40000pg/mL |

## 【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]

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