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-10、IL-12 p70、IL-6、IL-2、IFN-γ、IL-4、IL-8、TNF-α、IL-1β、IL-17A、IFN-α、IL-5 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	Size	Cat.No.	Storage of opened/reconstituted material
Coupled magnetic beads	Coupled human IL-10、IL-12 p70、IL-6、IL-2、IFN-γ、IL-4、IL-8、TNF-α、IL-1β、IL-17A、IFN-α、IL-5 antibody with magnetic beads.	1×500ul	RM59351	Store in the dark at 2-8°C
Standard Lyophilized	recombinant human IL-10、IL-12 p70、IL-6、IL-2、IFN-γ、IL-4、IL-8、TNF-α、IL-1β、IL-17A、IFN-α、IL-5 in a buffered protein base with preservatives;	2vials	RM59350	Aliquot and store at 2-8 °C in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Concentrated Biotin Conjugate Antibody (100×)	concentrated biotinylated antibody cocktail	1×50ul	RM59352	May be stored for up to 6 month at 2-8 °C.
Streptavidin-PE Concentrated (5×)	concentrated streptavidin-phycoerythrin conjugate with preservatives.	1×1ml	RM59400	
Standard/Sample Diluent (R1)	a buffered protein base with preservative.	1×20mL	RM59401	
Biotin-Conjugate Antibody Diluent (R2)	a buffered protein base with preservative.	1×12mL	RM59402	
Streptavidin-PE Diluent(R3)	a buffered protein base with preservative.	1×12mL	RM59403	
Wash Buffer(20x)	a buffered protein base with preservative.	1×30mL	RM59404	
Plate Sealers	4 Strips			
Specification	1			
Microplate	1			

Other Supplies Required

ABplex-100 analyzer with X-Y platform

1．Hand-held microplate magnet or platewasher with a magnetic platform

2．Multi-channel pipette,manifold dispenser,or automated dispensing unit

3．Horizontal orbital microplate shaker capable of maintaining a speed of 1200 ± 50 rpm

4．Microcentrifuge

5．Pipettes and pipette tips

6．Deionized or distilled water

7．Polypropylene test tubes for dilution of standards and samples

8．10 mL and 50 mL graduated cylinders

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

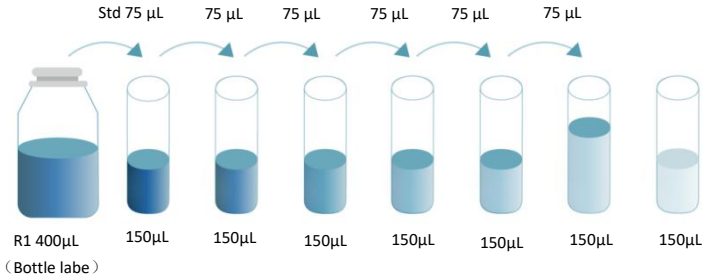
Bring all reagents to room temperature before use.If crystals have formed in the concentrate,Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.

Working Biotin Conjugate Antibody-Dilute 1:100 of Concentrated Biotin Conjugate Antibody (100x) with Biotin-Conjugate Antibody Diluent (R2) before use,for example:Add 20 µL of Concentrated Biotin Conjugate Antibody (100x) to 1980 µL Biotin-Conjugate Antibody Diluent (R2) to prepare 2000 µL Working Biotin Conjugate Antibody Buffer.

Working Streptavidin-PE-Dilute 1:5 of Concentrated Streptavidin-PE (5x) with Streptavidin-PE Diluent (R3) before use,for example:Add 200µL of Concentrated Streptavidin-PE (5x) to 800µL Streptavidin-PE Diluent (R3) to prepare 1000µL Working Streptavidin-PE Buffer.

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.

Standard-Reconstitute the Standard Lyophilized with Standard/Sample Diluent (R1).For the redissolution volume,see the label of the corresponding calibrator bottle.Allow the standard to sit for a minimum of 20 minutes prior to making dilutions.Prepare the tube containing R1 diluent according to the figure below,and perform gradient dilution according to the prompts.



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 Standard/Sample Diluent (R1).

Serum (pretreated) and plasma samples require a appropriate dilution with Standard/Sample Diluent (R1).

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 standards,and samples as directed in the previous sections.

2.Add 50 µL of standard or sample per well.

3.Resuspend the Coupled magnetic beads by inversion or vortexing. Add 5 µL of the microparticle cocktail 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 (0.12" orbit) set at 1200±50 rpm.

4.Using a magnetic device designed to accommodate a microplate,wash by applying the magnet to the bottom of the microplate,allow 2min before removing the liquid,filling each well with Wash Buffer (100µL) and allow 2min before removing the liquid again.Uniform removal of liquid is essential for good performance. Note:Do NOT blot;this may cause a loss of microparticles.

5.Add 50µL of Working Detection antibodies to all wells.Securely cover with a foil plate.sealer and incubate for 30min at 37℃ on the shaker set at 1200±50 rpm.

6.Repeat the wash as in step 4.

7.Add 50µL of Working Streptavidin-PE to all 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 as in step 4.

9.Resuspend the microparticles by adding 55µL of Wash Buffer to each well. Incubate for 2minutes on the shaker set at 1200±50 rpm.

10.Read within 90minutes using the ABplex-100 analyzer.

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

Assay Procedure Summary

Prepare the standard and reagents

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Add 50μL of standards or test samples to each well

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Add 5μL coded microspheres to each well

Incubate for 1 hour at 37℃,then wash 1times

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Add 50μL Working detection antibody

Incubate for 0.5 hour at 37℃,then wash 1times

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Add 50μL SA-PE

Incubate for 15min at 37℃ under dark condition

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Add 55μL wash buffer

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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

LOB-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 (LOB)
IL-1β	3.17pg/mL
IL-2	0.76pg/mL
IL-4	0.54pg/mL
IL-5	1.16pg/mL
IL-6	0.67pg/mL
IL-8	0.61pg/mL
IL-10	0.66pg/mL
IL-12 p70	0.78pg/mL
IL-17A	0.71pg/mL
IFN-α	0.51pg/mL
IFN-γ	2.53pg/mL
TNF-α	0.56pg/mL

Specificity

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	IL-13
IL-15	IL-18
IL-22	

Precision

Inter-Assay Precision-generated from the mean of the %CV's across two different concentrations of analytes across 25 different serum assays.

Analyte	Inter-Assay（%CV）
IL-1β	8.2
IL-2	3.4
IL-4	4.2
IL-5	2.6
IL-6	4.7
IL-8	4.5
IL-10	7.1
IL-12 p70	2.6
IL-17A	3.4
IFN-α	5.1
IFN-γ	5.4
TNF-α	3.8

Recovery

The data represents mean percent recovery of spiked standards ranging from low, medium, and high concentration in serum matrix samples.

Analyte	n	Recovery Average %
IL-1β	4	91.03
IL-2	4	82.91
IL-4	4	74.19
IL-5	4	72.33
IL-6	4	85.33
IL-8	4	90.55
IL-10	4	94.84
IL-12 p70	4	81.96
IL-17A	4	79.94
IFN-α	4	96.42
IFN-γ	4	91.74
TNF-α	4	83.76

Detection Range

Analyte	Bead	Concentration of Standard 1 (pg/mL)
IL-1β	12	5000pg/mL
IL-2	15	1000pg/mL
IL-4	16	500pg/mL
IL-5	21	1000pg/mL
IL-6	22	1000pg/mL
IL-8	24	2000pg/mL
IL-10	25	1000pg/mL
IL-12 p70	31	5000pg/mL
IL-17A	33	5000pg/mL
IFN-α	35	500pg/mL
IFN-γ	42	3000pg/mL
TNF-α	44	1000pg/mL