ClinMax™ Human IL-12p70 ELISA Kit

Catalog Number：CRS-B009

Pack Size: 96 tests

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

INTENDED USE

The kit is developed for quantitative detection of IL-12p70 in human serum and cell culture supernates. It is intended for research use only (RUO).

BACKGROUND

Interleukin 12 (IL-12) is an interleukin that is naturally produced by dendritic cells, macrophages, neutrophils, and human B-lymphoblastoid cells (NC-37) in response to antigenic stimulation. IL12 is a heterodimeric cytokine encoded by two separate genes, IL-12A (p35) and IL-12B (p40). The active heterodimer (referred to as 'p70'), and a homodimer of p40 are formed following protein synthesis.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of human Interleukin-12p70 (IL-12p70) by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-IL-12 Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-IL-12 Antibody to the plate and form Antibody-antigen-biotinylated antibody complex, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450nm and 630nm. The OD Value reflects the amount of IL-12p70 bound.

PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. The kit is suitable for cell supernatant, serum and plasma samples.
3. Do not use reagents past their expiration date.
4. Do not mix or substitute reagents with those from other kits or other lot number kits.
5. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell supernatant samples need step dilution, except for the final dilution with diluent, other intermediate dilutions can be in cell culture medium.
6. Differences in test results can be caused by a variety of factors, including laboratory operator,

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pipette usage, plate washing technique, reaction time or temperature, and kit storage.

7. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

MATERIALS PROVIDED

Table1. Materials provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
CRB009-C01	Pre-coated Anti-IL-12 Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
CRB009-C02	Human IL-12p70 Standard	20 μg×2	Powder	2-8°C	-70°C
CRB009-C03	Biotin-Anti-IL-12 Antibody Con. Solution	100 μL	Liquid	2-8°C	2-8°C
CRB009-C04	Biotin-Antibody Dilution Buffer	8 mL	Liquid	2-8°C	2-8°C
CRB009-C05	Streptavidin-HRP Con. Solution	500 μL	Liquid	2-8°C, avoid light	2-8°C, avoid light
CRB009-C06	Streptavidin-HRP Dilution Buffer	15 mL	Liquid	2-8°C	2-8°C
CRB009-C07	20× Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
CRB009-C08	1× Dilution Buffer	15 mL×2	Liquid	2-8°C	2-8°C
CRB009-C09	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
CRB009-C10	Stop Solution	6 mL	Liquid	2-8°C	2-8°C

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KIT STORAGE AND EXPIRATION DATE

1. The unopened kit is stable for 24 months from the date of manufacture if stored at 2°C to 8°C.
2. The opened kit should be stored per Table 1. The shelf life is 30 days from the date of opening.

Note: *a. Do not use reagents past their expiration date.*

b. Find the expiration date on the outside packaging.

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or multi-channel micropipettes and pipette tips: need to meet 10 μL, 300 μL, 1000 μL injection;

37ºC Incubator;

Single or dual wavelength microplate reader with 450nm and 630nm filter;

Tubes: 1.5 mL,10 mL;

Timer;

Reagent bottle;

Deionized or distilled water.

REAGENT PREPARATION

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

According to Table 2, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vortexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, and the size of the aliquot should not be less than 10 μg.

Table 2. Preparation method

Catalog	**Components**	**Size (96 T)**	**Storage solution concentration.**	**Reconstituted water Vol.**
CRB009-C02	Human IL-12p70 Standard	20 μg	20 μg/mL	1000 μL

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RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer

Dilute 50 mL 20×Washing Buffer with deionized or ultrapure water to 1000 mL.

1.2 Preparation of Biotin-Anti-IL-12p70 Antibody Solution

Prepare Biotin-Anti-IL-12p70 Antibody Solution by diluting 60 µL of Biotin-Anti-IL-12p70 Antibody Con. Solution into 6 mL Biotin-Antibody Dilution Buffer, mix gently well. The solution was freshly prepared just before use.

1.3 Preparation of IL-12p70 Streptavidin-HRP Solution

Prepare IL-12p70 Streptavidin-HRP Solution by diluting 300 µL of IL-12p70 Streptavidin-HRP Con. Solution into 12 mL Streptavidin-HRP Dilution Buffer, mix gently well. The solution was freshly prepared just before use.

2. Preparation of Standard curve

The concentration of the reconstituted human IL-12p70 Standard（CRB009-C02）is 20 µg/mL，prepare Cm by diluting 5 µL of the reconstituted human IL-12p70 Standard into 995 µL Sample Dilution Buffer, mix gently well. Label 8 tubes, one for each standard point: Std.-1, Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7, Std.-8. According to the following dilution scheme: 5 µL IL-12p70 Cm + 995 µL Sample Dilution Buffer. Shake gently to mix, labeled Std.-1 (Std.-1 = 500 pg/mL). Prepare 1:1 serial dilutions for the standard curve as follows: Pipette 500 µL of Sample Dilution Buffer into each tube. Pipette 500 µL of diluted standard (concentration of standard = 500 pg/mL) into the first tube, labeled Std.-2 (Std.-2 = 250 pg/mL), and mix. Pipette 500 µL of this dilution into the second tube, labeled Std.-3, and mix thoroughly before the next transfer. Sample Dilution Buffer serves as blank.

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Tubes/ Solution Code	Human IL-12p70 stock solution	Cm	Std.-1	Std.-2	Std.-3	Std.-4	Std.-5	Std.-6	Std.-7	Std.-8
Operating	5 µL	5 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
Solution Con.	20 µg/mL	100 ng/mL	500 pg/mL	250 pg/mL	120 pg/mL	62.5 pg/mL	31.25 pg/mL	15.625 pg/mL	7.8125 pg/mL	3.90625pg/mL
Dilution Buffer Vol.		995 µL	995 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL

3. Add Samples and Biotin-Antibody Solution

Add 50 µL IL-12p70 Standard to each well, or add 50 µL samples to each well, finally add 50 µL Biotin-Anti-IL-12p70 Antibody Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for 1.0 h.

4. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, gently tap the plate for 1 min, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for five times.

5. Add IL-12p70 Streptavidin-HRP Solution

For all wells, add 100 µL IL-12p70 Streptavidin-HRP Solution. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for 30 min, avoid light.

6. Washing

Repeat step 4.

7. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for 15 min, avoid light.

8. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

Note: *the color in the wells should change from blue to yellow.*

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9. Data Recording

Read the absorbance at 450nm and 630nm using UV/Vis microplate spectrophotometer.

***Note**: To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

CALCULATION OF RESULTS

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.).
2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.
3. Normal range of Standard curve: $R^2 \geq 0.9900$.
4. Detection range: 3.90 pg/mL-500 pg/mL. If the OD value of the sample to be tested is higher than 500 pg/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 3.90 pg/mL, the sample should be reported.

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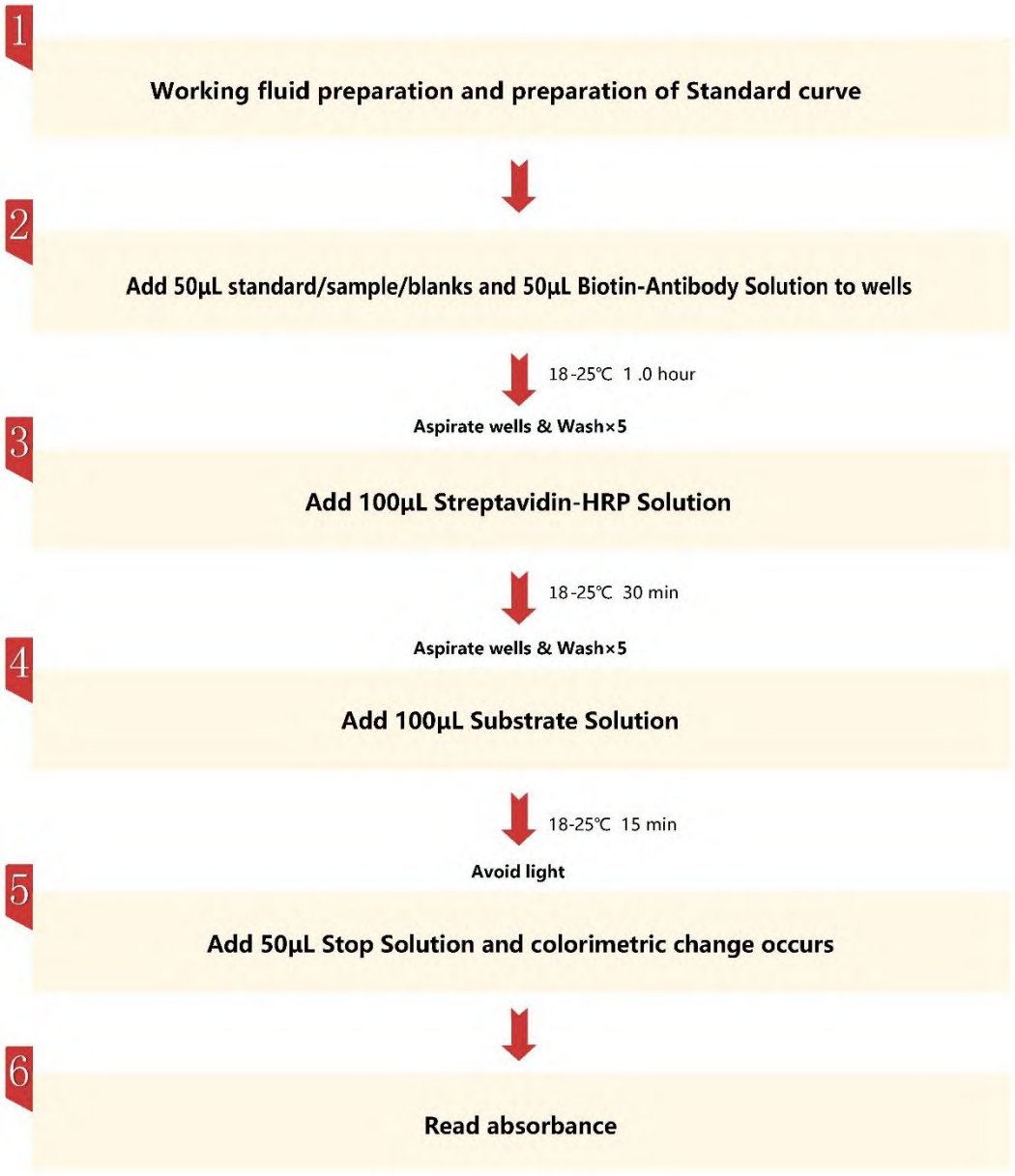
QUICK GUILD



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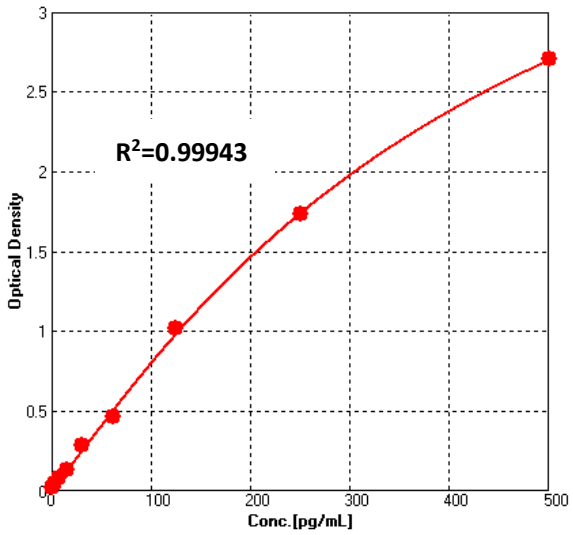
TYPICAL DATA

The following data is for reference only. The sample concentration was calculated based on the results of the standard curve.

IL-12p70 Standard (pg/mL)	$OD_{450nm-630nm}$
500	2.703
250	1.731
125	1.021
62.5	0.468
31.25	0.282
15.62	0.136
7.81	0.082
3.90	0.043
Blank	0.019



SENSITIVITY

The minimum detectable concentration of human IL-12p70 is 2.0 pg/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

PRECISION

1. Intra-assay Precision

Three samples of known concentration were tested ten times on one plate to assess intra-assay precision.

2. Inter-assay Precision

Three samples of known concentration were tested on three different plates, ten replicates in each plate to assess inter-assay precision.

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	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	30	30	30
Mean (pg/mL)	515.00	412.00	282.50	504.33	420.53	259.42
SD	29.92	15.11	9.51	25.78	21.03	11.37
CV (%)	5.81	3.67	3.37	5.11	5.00	4.38

***Note**: The example data is for reference only.*

RECOVERY

Three parts of blank serum were added with different concentrations of human IL-12p70, and the serum without human IL-12p70 was used as background to calculate the recovery rate. The Range of the recovery rate is 85.98%-113.32%, and the average recovery is 103.94%.

Sample Type	Average% Recovery	Range
Serum(n=5)	103.94	85.98-113.32%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human IL-12p70 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Serum
1:2	Average Recovery (%)	98.33
	Range (%)	96.80-101.10
1:4	Average Recovery (%)	110.08
	Range (%)	106.58-114.04
1:8	Average Recovery (%)	108.49
	Range (%)	103.92-112.90

***Note**: The example data is for reference only.*

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SPECIFICITY

This assay recognizes natural and recombinant human IL-12p70. No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines.

Human	
IL-1β	IL-10
IL-2	IL-15
IL-4	IL-21
IL-6	GM-CSF
IL-7	TNF-alpha

SAMPLE VALUES

80 human healthy sample were evaluated for the presence of human IL-12p70 in this assay. No medical histories were available for the donors used in this study.

Sample	n	Concentration (pg/mL)	Detection percentage (%)	Detection average Concentration (pg/mL)
Serum	80	n.d. – 18.90	45.00	7.12

Note: n.d. means that the concentration value cannot be detected which is lower than the sensitivity. The example data is for reference only.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human IL-12 (95/544). Reference Reagent is calibrated by NIBSC/WHO in April 2013.

NIBSC/WHO (95/544) approximate value (U/mL) = 0.1 × Human IL-12p70 value (pg/mL)

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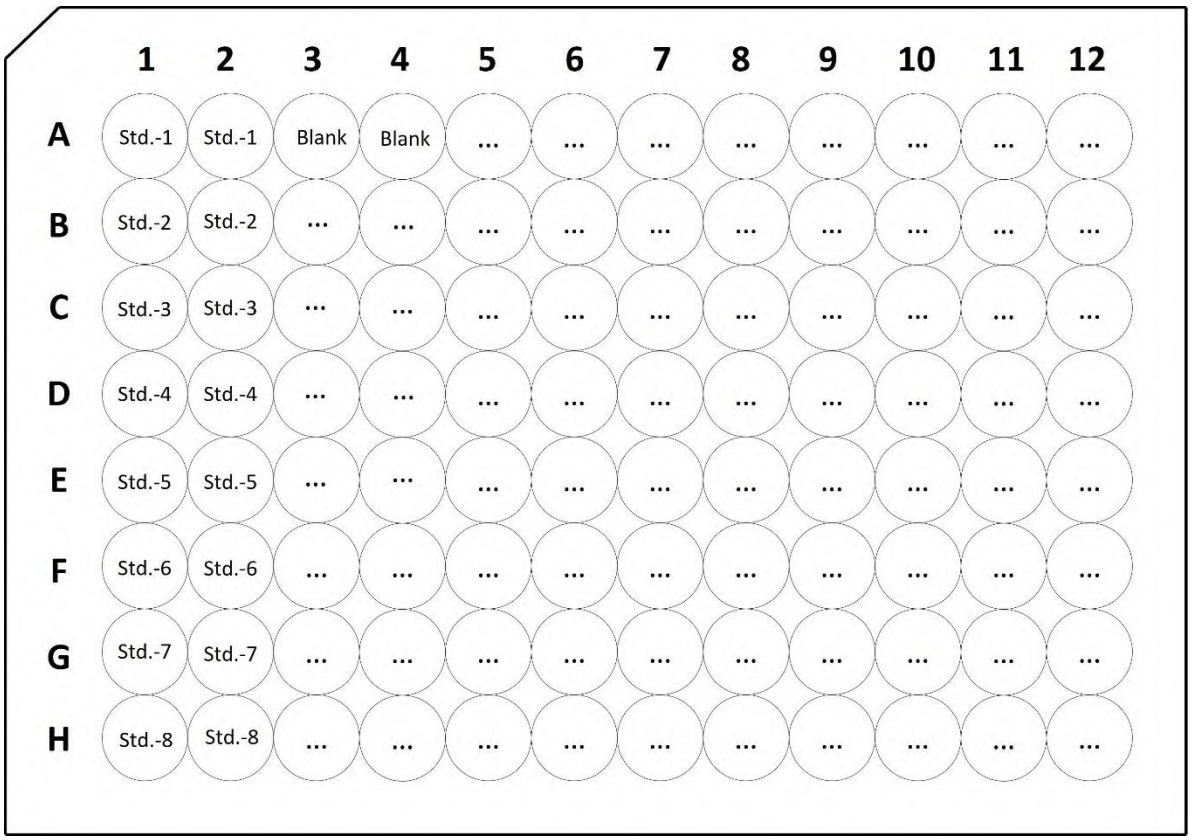
PLATE LAYOUT



Note: Blank is a Blank Dilution Buffer hole.

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TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

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