



## **Human Interleukin-10 (IL-10) ELISA Kit (Enzyme-Linked Immunosorbent Assay)**

Catalog Number: CRS-B005

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-10 in human serum and cell culture supernates. It is intended for research use only (RUO).

## BACKGROUND

Interleukin 10 (IL-10), also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine. IL-10 is a cytokine with multiple, pleiotropic, effects in immunoregulation and inflammation. It downregulates the expression of  $T_H1$  cytokines, MHC class II antigens, and co-stimulatory molecules on macrophages. It also enhances B cell survival, proliferation, and antibody production. IL-10 can block NF- $\kappa$ B activity and is involved in the regulation of the JAK-STAT signaling pathway.

## PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of human Interleukin-10 (IL-10) by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-IL-10 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-10 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-10 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, 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<br>(96 tests) | Format | Storage            |                    |
|------------|-------------------------------------------|--------------------|--------|--------------------|--------------------|
|            |                                           |                    |        | Unopened           | Opened             |
| CRS005-C01 | Pre-coated Anti-IL-10 Antibody Microplate | 1 plate            | Solid  | 2-8°C              | 2-8°C              |
| CRS005-C02 | Human IL-10 Standard                      | 60 µg×2            | Powder | 2-8°C              | -70°C              |
| CRS005-C03 | Biotin-Anti-IL-10 Antibody Con. Solution  | 100 µL             | Liquid | 2-8°C              | -70°C              |
| CRS005-C04 | Biotin-Antibody Dilution Buffer           | 8 mL               | Liquid | 2-8°C              | 2-8°C              |
| CRS005-C05 | Streptavidin-HRP Con. Solution            | 0.5 mL             | Liquid | 2-8°C, avoid light | 2-8°C, avoid light |
| CRS005-C06 | Streptavidin-HRP Dilution Buffer          | 15 mL              | Liquid | 2-8°C              | 2-8°C              |
| CRS005-C07 | 20× Washing Buffer                        | 50 mL              | Liquid | 2-8°C              | 2-8°C              |
| CRS005-C08 | 1× Dilution Buffer                        | 15 mL×2            | Liquid | 2-8°C              | 2-8°C              |
| CRS005-C09 | Substrate Solution                        | 12 mL              | Liquid | 2-8°C, avoid light | 2-8°C, avoid light |
| CRS005-C10 | Stop Solution                             | 6 mL               | Liquid | 2-8°C              | 2-8°C              |

**NOTE: Bubbles in microplate wells do not affect the experiment and require no action. Proceed with the experimental procedures and methods described below.**

## 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;  
37°C 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°C-25°C) before use. If crystals have formed in buffer solution, place the sample in an 37°C 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 5 µg.

**Table 2. Preparation method**

| Catalog    | Components           | Size (96 T) | Storage solution concentration. | Reconstituted water Vol. |
|------------|----------------------|-------------|---------------------------------|--------------------------|
| CRS005-C02 | Human IL-10 Standard | 60 µg       | 60 µg/mL                        | 1000 µL                  |

## 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-10 Antibody Solution

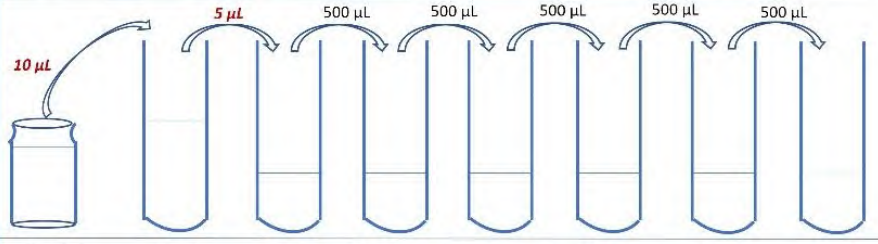
Prepare Biotin-Anti-IL-10 Antibody Solution by diluting 60 µL of Biotin-Anti-IL-10 Antibody Con. Solution into 6 mL Biotin-Antibody Dilution Buffer, mix gently well. The solution was freshly prepared just before use.

#### 1.3 Preparation of IL-10 Streptavidin-HRP Solution

Prepare IL-10 Streptavidin-HRP Solution by diluting 150 µL of IL-10 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-10 Standard (CRS005-C02) is 60 µg/mL, prepare Cm by diluting 10 µL of the reconstituted human IL-10 Standard into 990 µL Sample Dilution Buffer, mix gently well. Label 6 tubes, one for each standard point: Std.-1, Std.-2, Std.-3, Std.-4, Std.-5, Std.-6. According to the following dilution scheme: 5 µL IL-10 Cm + 2995 µL Sample Dilution Buffer. Shake gently to mix, labeled Std.-1 (Std.-1 = 1000 pg/mL). Prepare 1:2 serial dilutions for the standard curve as follows: Pipette 1000 µL of Sample Dilution Buffer into each tube. Pipette 500 µL of diluted standard (concentration of standard = 800 pg/mL) into the first tube, labeled Std.-2 (Std.-2 = 333.33 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.

| Tubes/<br>Solution<br>Code | Human IL-10<br>stock solution                                                      | Cm        | Std.-1     | Std.-2       | Std.-3       | Std.-4      | Std.-5      | Std.-6     |
|----------------------------|------------------------------------------------------------------------------------|-----------|------------|--------------|--------------|-------------|-------------|------------|
| Operating                  |  |           |            |              |              |             |             |            |
| Solution<br>Con.           | 60 µg/mL                                                                           | 600 ng/mL | 1000 pg/mL | 333.33 pg/mL | 111.11 pg/mL | 37.04 pg/mL | 12.35 pg/mL | 4.12 pg/mL |
| Dilution<br>Buffer Vol.    |                                                                                    | 990 µL    | 2995 µL    | 1000 µL      | 1000 µL      | 1000 µL     | 1000 µL     | 1000 µL    |

### 3. Add Samples and Biotin-Antibody Solution

Add 50 µL IL-10 Calibrator to each well, or add 50 µL samples to each well, finally add 50 µL Biotin-Anti-IL-10 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-10 Streptavidin-HRP Solution

For all wells, add 100 µL IL-10 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.*

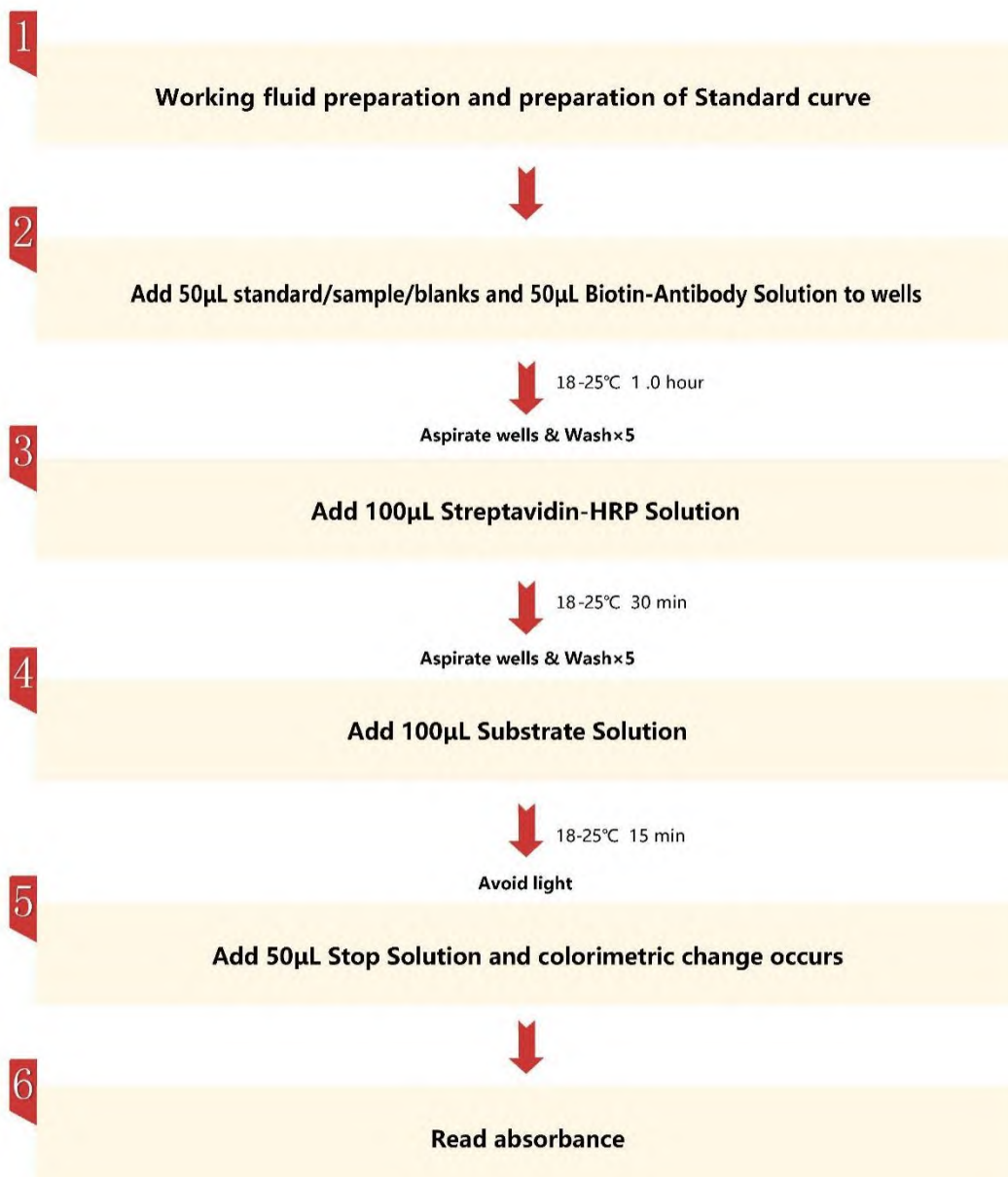
## 9. Data Recording

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.*

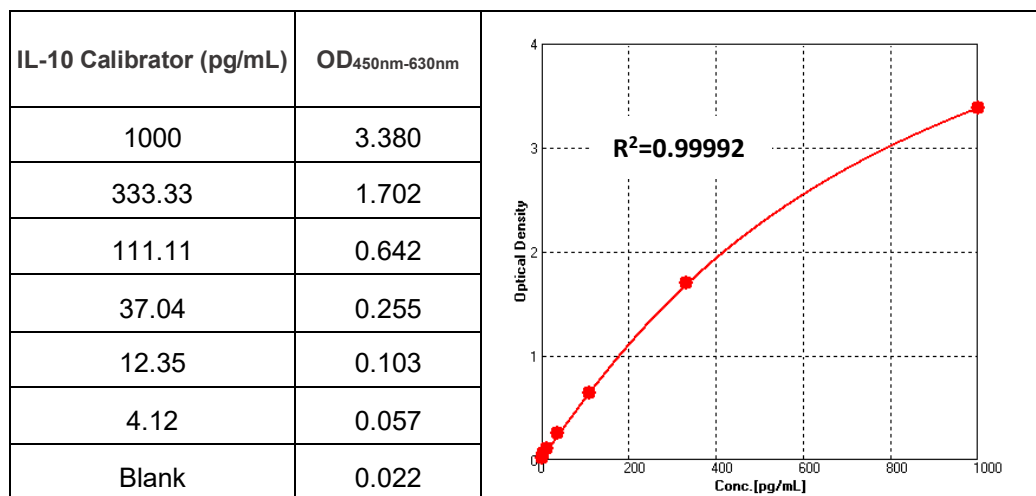
## CALCULATION OF RESULTS

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: 4.12 pg/mL-1000 pg/mL. If the OD value of the sample to be tested is higher than 1000 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 4.12 pg/mL, the sample should be reported.

**QUICK GUILD**

## TYPICAL DATA

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



## SENSITIVITY

The minimum detectable concentration of human IL-10 is 1.87 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.

|              | Intra-assay Precision |        |        | Inter-assay Precision |        |        |
|--------------|-----------------------|--------|--------|-----------------------|--------|--------|
| Sample       | 1                     | 2      | 3      | 1                     | 2      | 3      |
| n            | 10                    | 10     | 10     | 30                    | 30     | 30     |
| Mean (pg/mL) | 892.76                | 739.64 | 426.87 | 891.79                | 810.95 | 513.08 |
| SD           | 43.72                 | 25.63  | 17.24  | 38.74                 | 39.39  | 23.18  |
| CV (%)       | 4.90                  | 3.46   | 4.04   | 4.34                  | 4.86   | 4.52   |

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

## RECOVERY

The serums were added with different concentrations of human IL-10, and the serum without human IL-10 was used as background to calculate the recovery rate. The Range of the recovery rate is 86.64%-112.00%, and the average recovery is 98.63%.

| Sample Type | Average% Recovery | Range         |
|-------------|-------------------|---------------|
| Serum(n=5)  | 98.63             | 86.64-112.00% |

## LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human IL-10 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

|     |                      | Serum        |
|-----|----------------------|--------------|
| 1:2 | Average Recovery (%) | 100.48       |
|     | Range (%)            | 99.80-113.00 |
| 1:4 | Average Recovery (%) | 96.86        |
|     | Range (%)            | 86.73-110.46 |
| 1:8 | Average Recovery (%) | 95.13        |
|     | Range (%)            | 88.78-110.21 |

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

## SPECIFICITY

This assay recognizes natural and recombinant human IL-10. No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines.

| Human        |               |
|--------------|---------------|
| IL-1 $\beta$ | IL-15         |
| IL-2         | IL-21         |
| IL-4         | GM-CSF        |
| IL-6         | TNF-alpha     |
| IL-7         | IFN- $\gamma$ |

## SAMPLE VALUES

80 human healthy sample were evaluated for the presence of human IL-10 in this assay. No medical histories were available for the donors used in this study.

| Sample | n  | Concentration<br>(pg/mL) | Detection percentage (%) | Detection average Concentration<br>(pg/mL) |
|--------|----|--------------------------|--------------------------|--------------------------------------------|
| Serum  | 80 | n.d. – 46.83             | 99.17                    | 16.62                                      |

*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-10 (93/722). Reference Reagent is calibrated by NIBSC/WHO in April 2013.

NIBSC/WHO (93/722) approximate value (U/mL) = 0.005  $\times$  Human IL-10 value (pg/mL)

## PLATE LAYOUT

|   | 1      | 2      | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  |
|---|--------|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A | Std.-1 | Std.-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| B | Std.-2 | Std.-2 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| C | Std.-3 | Std.-3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| D | Std.-4 | Std.-4 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| E | Std.-5 | Std.-5 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| F | Std.-6 | Std.-6 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| G | Blank  | Blank  | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| H | ...    | ...    | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

*Note: Blank is a Blank Dilution Buffer hole.*

## TROUBLESHOOTING GUIDE

| Problem                                                     | Cause                                                            | Solution                                                                                                                                                                                                                                                               |
|-------------------------------------------------------------|------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Poor standard curve                                         | * Inaccurate pipetting                                           | * Check pipettes                                                                                                                                                                                                                                                       |
| Large CV                                                    | * Inaccurate pipetting<br>* Air bubbles in wells                 | * Check pipettes<br>* Remove bubbles in wells                                                                                                                                                                                                                          |
| High background                                             | * Plate is insufficiently washed<br>* Contaminated wash buffer   | * Review the manual for proper wash.<br>* Make fresh wash buffer                                                                                                                                                                                                       |
| Very low readings across the plate                          | * Incorrect wavelengths<br>* Insufficient development time       | * Check filters/reader<br>* 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<br>* Reagents not at room temperature | * Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay<br>* Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts |