Version 23-04/24

User's Manual

Human Interleukin 10 Receptor, Beta, IL10RB ELISA Kit

REF **DEIA256**

Σ **5 plates**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The human IL10RB / CRFB4 ELISA Pair Set is for the quantitative determination of human IL10RB / CRFB4.

This ELISA Pair Set contains the basic components required for the development of sandwich ELISAs.

General Description

The functional IL-10 receptor consists of two polypeptide chains, a ligand binding chain IL-10 Rα (IL-10 R1) and a signal transduction molecule known as IL-10 Rβ (IL-10 R2). Both chains of the IL-10 Rare expressed on many hematopoietic cells, including lymphocytes, monocytes and neutrophils, with the IL-10 Rβ chain expressed at higher levels than the IL-10 Rα chain in most cases. Interleukin 10 receptor beta (IL-10 Rβ), also known as CD210b and CRF2-4, is an 80-85 kDa member of the type II cytokine receptor family of proteins and contains two fibronectin type-III domains. The widely expressed IL-10 Rβ serves as a signal transducing accessory chain not only for IL-10, but also for other interleukins including IL-22, -28A, -28B and -29 (plus IL-26 in human) when complexed to the ligand-binding chains. It has been shown that the action of IL-10 delivered through its receptor can affect a variety of immune functions including inhibition of proinflammatory cytokine synthesis and regulation of the growth and function of B cells and antigen presenting cells. The human IL-10 Rβ shares 69% homology to its mouse counterpart in amino acid sequence. Research evidences indicated that defects in IL10RB are associated with susceptibility to hepatitis B virus infection.

Principles of Testing

The CD ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for IL10RB / CRFB4 coated on a 96-well plate. Standards and samples are added to the wells, and any IL10RB / CRFB4 present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated rabbit anti-IL10RB polyclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". The wells are again washed and TMB substrate solution is loaded, which produces color in proportion to the amount of IL10RB / CRFB4 present in the sample. To end the enzyme reaction, the stop solution is added and absorbances of the microwell are read at 450 nm.

Reagents And Materials Provided

Bring all reagents to room temperature before use.

Capture Antibody: 0.5 mg/mL of mouse anti-IL10RB monoclonal antibody. Dilute to a working concentration of 2.0 µg/mL in CBS before coating.

Detection Antibody: 0.7 mg/mL rabbit anti-IL10RB polyclonal antibody conjugated to horseradish-peroxidase (HRP). Dilute to a working concentration of 0.5 µg/mL in detection antibody diluteion buffer before use

Standard: Each vial contains 110 ng of recombinant IL10RB. Reconstitute standard powder with 1mL detection antibody dilution buffer. After reconstitution, store at -20°C to -80°C in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 5000

pg/mL is recommended.

Materials Required But Not Supplied

CBS: 0.05M Na_2CO_3, $NaHCO_3$, pH 9.6, 0.2 µm filtered

TBS: 25mM Tris, adjust pH to 7.4 by HCl

Wash Buffer: 0.05% Tween20 in TBS, pH 7.2-7.4

Blocking Buffer: 2% BSA in Wash Buffer

Sample dilution buffer: 0.1% BSA in wash buffer, pH 7.2-7.4, 0.2 µm filtered

Detection antibody dilution buffer: 0.5% BSA in wash buffer, pH 7.2-7.4, 0.2 µm filtered

Substrate Solution: To achieve best assay results, fresh substrate solution is recommended

Substrate stock solution: 10mg / ml TMB (Tetramethylbenzidine) in DMSO

Substrate dilution buffer: 0.05M Na_2HPO_4 and 0.025M citric acid; adjust pH to 5.5

Substrate working solution: For each plate dilute 250 µl substrate stock solution in 25 ml substrate dilution buffer and then add 80 µl 0.75% H_2O_2, mix it well

Stop Solution: 2 N H_2SO_4

Storage

Capture Antibody: Aliquot and store at -20°C to -80°C for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Detection Antibody: Store at 4°C and protect it from prolonged exposure to light for up to 6 months from date of receipt. Do not freeze!

Standard: Store lyophilized standard at -20°C to -80°C for up to 6 months from date of receipt. Aliquot and store the reconstituted standard at -80°C for up to 1 month. Avoid repeated freezethaw cycles.

Plate Preparation

1. Dilute the capture antibody to the working concentration in CBS. Immediately coat a 96-well microplate with 100µL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4°C.
2. Aspirate each well and wash with at least 300µl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100 µL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at

room temperature.

2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 µL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 200 µL of substrate solution to each well. Incubate for 20 minutes at room temperature (if substrate solution is not as requested, the incubation time should be optimized). Avoid placing the plate in direct light.
6. Add 50 µL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

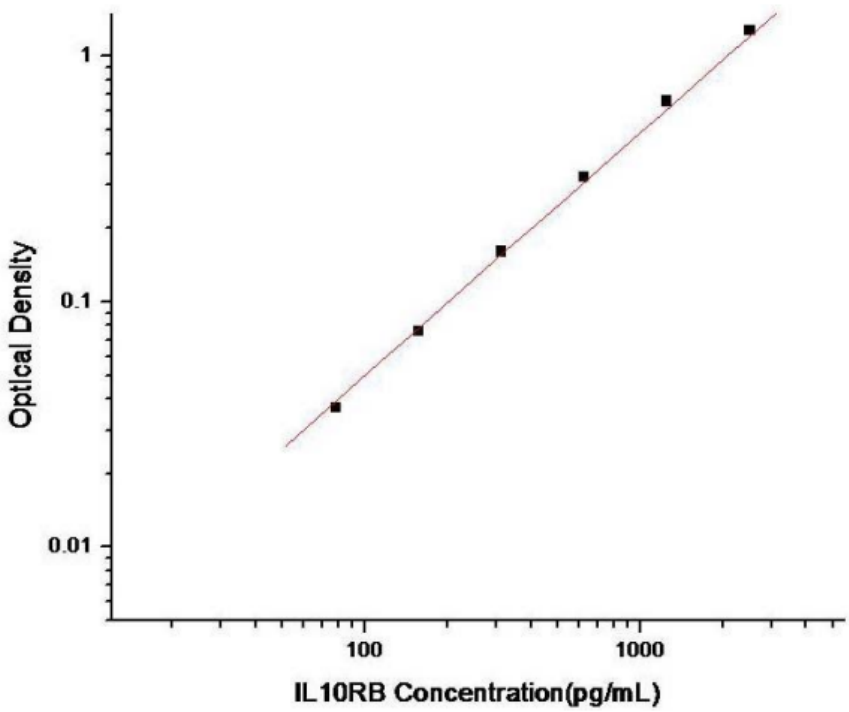
Calculation

1. Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
2. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
3. To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
4. Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

Typical Standard Curve

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.

Concentration (pg/ml)	Zero standard subtracted OD
0	0.000
78.125	0.037
156.25	0.076
312.5	0.160
625	0.322
1250	0.657
2500	1.275
5000	2.137



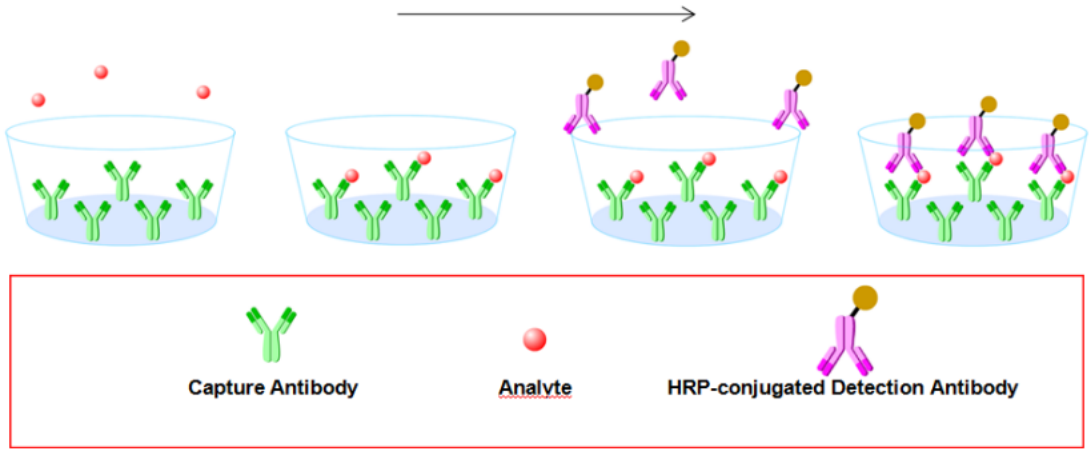
Sensitivity

The minimum detectable dose of human IL10RB / CRFB4 was determined to be approximately **78.125 pg/ml** .

This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

Precautions

The Stop Solution suggested for use with this Pair Set is an acid solution. Wear eye, hand, face, and clothing protection when using this material.



Trouble Shooting

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 ℃
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated.	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples