Human CLEC4A / CLECSF6 / DCIR ELISA Pair Set

Catalog Number : SEK11476

To achieve the best assay results, this manual must be read carefully before using this product and the assay is run as summarized in the General ELISA protocol.

BACKGROUND

Dendritic cell immunoreceptor (DCIR), also known as C-type lectin domain family 4 member A (CLEC4A), C-type lectin superfamily member 6 (CLECSF6), is a single-pass type II C-type lectin receptor expressed mainly in dendritic cells (DCs), which is a negative regulator of DC expansion and has a crucial role in maintaining the homeostasis of the immune system. The Dectin-2 family of C-type lectins that includes Dectin-2, BDCA-2, DCIR, DCAR, Clecsf8 and Mincle. These type II receptors contain a single extracellular carbohydrate recognition domain and have diverse functions in both immunity and homeostasis. DCIR is the only member of the family which contains a cytoplasmic signalling motif and has been shown to act as an inhibitory receptor, while BDCA-2, Dectin-2, DCAR and Mincle all associate with FcRgamma chain to induce cellular activation, including phagocytosis and cytokine production. Dectin-2 and Mincle have been shown to act as pattern recognition receptors for fungi, while DCIR acts as an attachment factor for HIV. In addition to pathogen recognition, DCIR has been shown to be pivotal in preventing autoimmune disease by controlling dendritic cell proliferation. DCIR expressed on antigen presenting cells and granulocytes and acts as an inhibitory receptor via an intracellular immunoreceptor tyrosine-based inhibitory motif (ITIM). It may also be involved via its ITIM motif in the inhibition of B-cell-receptor-mediated calcium mobilization and protein tyrosine phosphorylation. Additionally, DCIR can participate in the capture of HIV-1 and promote infection in trans and in cis of autologous CD4(+) T cells from human immature monocyte-derived DCs. DCIR acts as a ligand for HIV-1 and is involved in events leading to productive virus infection.

PRINCIPLE OF THE TEST

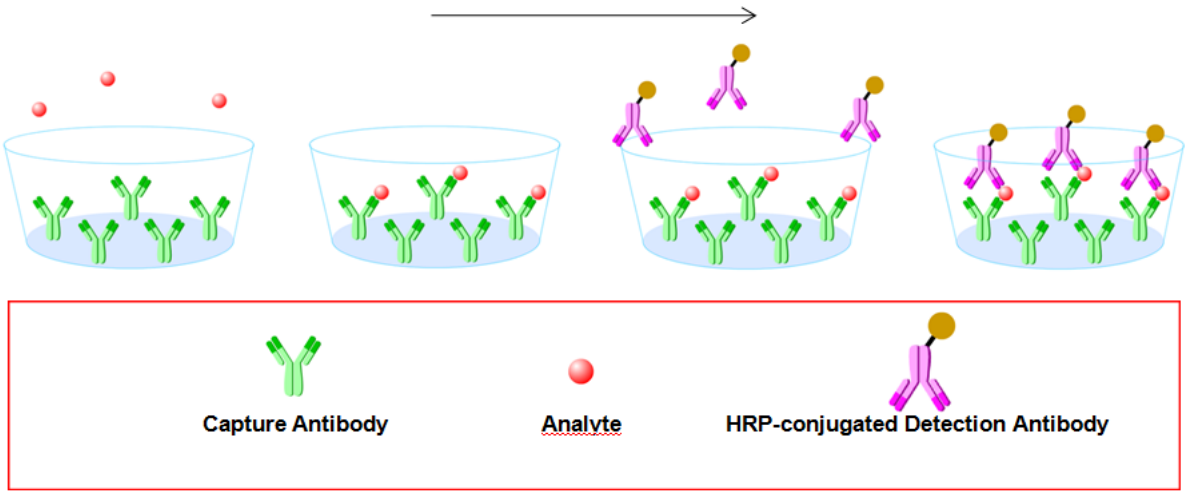
The Sino Biological ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for DCIR / CLEC4A / CLECSF6 coated on a 96-well plate. Standards and samples are added to the wells, and any DCIR / CLEC4A / CLECSF6 present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated mouse anti-DCIR / CLEC4A / CLECSF6 monoclonal 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 DCIR / CLEC4A / CLECSF6 present in the sample. To end the enzyme reaction, the stop solution is added and absorbances of the microwell are read at 450 nm.

INTENDED USE

◆ The human DCIR / CLEC4A / CLECSF6 ELISA Pair Set is for the quantitative determinationof human DCIR / CLEC4A / CLECSF6.

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

ASSAY PROCEDURE SUMMARY



This Pair Set has been configured for research use only and is not to be used in diagnostic procedures.

MATERIALS PROVIDED

Bring all reagents to room temperature before use.

Capture Antibody – 0.2 mg/mL of mouse anti-CLEC4A monoclonal antibody, Dilute to a working concentration of 2.0 µg/mL in CBS before coating. (Catalog: # 11476-MM04)

Detection Antibody – 0.5 mg/mL mouse anti-CLEC4A monoclonal antibody conjugated to horseradish-peroxidase (HRP). Dilute to working concentration of 0.5 µg/mL in detection antibody dilution buffer before use. (Catalog: # 11476-MM02)

Standard – Each vial contains 45 ng of recombinant CLEC4A. Reconstitute standard powder with 1 mL detection antibody dilution buffer. After reconstitution, store at -20℃ to -80℃ in a manual defrost freezer. A seven-point standard curve usi ng 2-fold serial dilutions in sample dilution buffer, and a high standard of 600 pg/mL is recommended.

SOLUTIONS REQUIRED

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

TBS - 20 mM Tris, 150 mM NaCl, pH 7.4

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 25ml substrate dilution buffer and then add 80 µl 0.75% H_2O_2 , mix it well

Stop Solution - 2 N H_2SO_4

PRECAUTION

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.

STORAGE

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

Detection Antibody: Protect it from prolonged exposure to light. Aliquot and store at -20℃ to -80℃ and for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

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

GENERAL ELISA PROTOCOL

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℃.
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

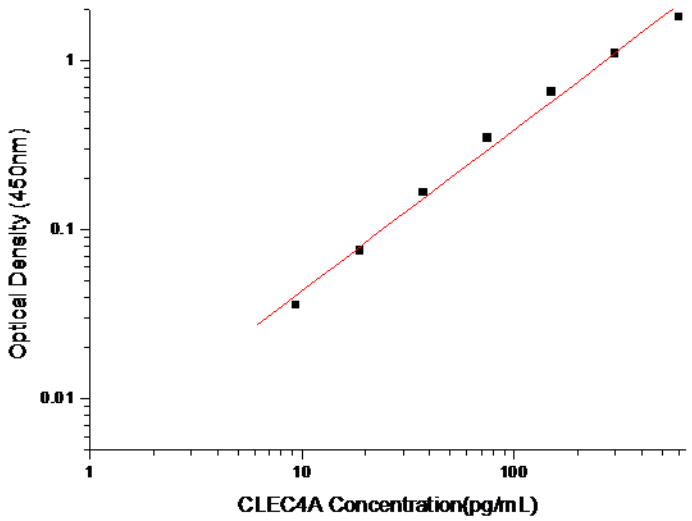
1.Add 100 μL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2.Repeat the aspiration/wash as in step 2 of plate preparation.
3.Add 100 μL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and 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 OF RESULTS

●Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
●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.
●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.
●Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

TYPICAL DATA

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
9.375	0.036
18.75	0.075
37.5	0.167
75	0.349
150	0.654
300	1.105
600	1.800

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of Human DCIR / CLEC4A / CLECSF6 was determined to be approximately 9.375 pg/ml. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

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

ELISA Plate Template

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

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Notes