Sino Biological Inc.
Biological Solution Specialist

Human CD48 ELISA Pair Set

(BCM1 / SLAMF2)

Catalog Number : SEK10797

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

CD48, also known as SLAMF2, BCM-1 and BLAST-1, is a GPI-linked protein belonging to the CD2 subfamily of immunoglobulin superfamily molecules. In rodents, CD48 is the ligand for CD2 whereas in humans, CD58 is the ligand for CD2. Human CD48 cDNA encodes a 243 aa precursor that includes a 26 aa signal sequence, a 194 aa mature protein that contains one Ig-like V-type domain and one Ig-like C2-type domain, and a 23 aa C-terminal propeptide. It has a high degree of sequence homology to CD58 (LFA-3), and human CD48 shares approximately 50% aa sequence identity with mouse and rat CD48. CD48 is expressed on most lineage-committed hematopoietic cells but not on hematopoietic stem cells or multipotent hematopoietic progenitors. CD48 performs biological functions in a variety processes including adhesion, pathogen recognition, cellular activation, and cytokine regulation, and emerges as a critical effector molecule in immune responses.

Assessment of CD48 expression in a murine model of experimental asthma revealed that CD48 is induced by allergen challenge and partially regulated by IL-3. Additionally, anti-IL-3 reduces CD48 expression and the degree of airway inflammation. Thus, CD48 is an IL-3-induced activating receptor on eosinophils, likely involved in promoting allergic inflammation. CD48 reorganization was vital for T cell IL-2 production by mediating lateral association of the early signaling component linker for activated T cells (LAT) to the TCR/CD3 complex. CD48 in turn shuttles the transmembrane adapter molecule LAT. CD48 is known to be virtually expressed by all human leukocytes. Its ligand, 2B4, is a signaling lymphocyte activation molecule-related receptor involved in NK cell activation. CD48 expression in dendritic cells (DCs) affects NK cell functions during NK/DC cross-talk, because NK cells obtained from normal donors and from X-linked lymphoproliferative disease patients are, respectively, triggered or inhibited by DCs expressing surface CD48. Remarkably, IFN-gamma production by lymph node NK cells, in contrast to blood NK cells, can be negatively modulated by 2B4/CD48 interactions.

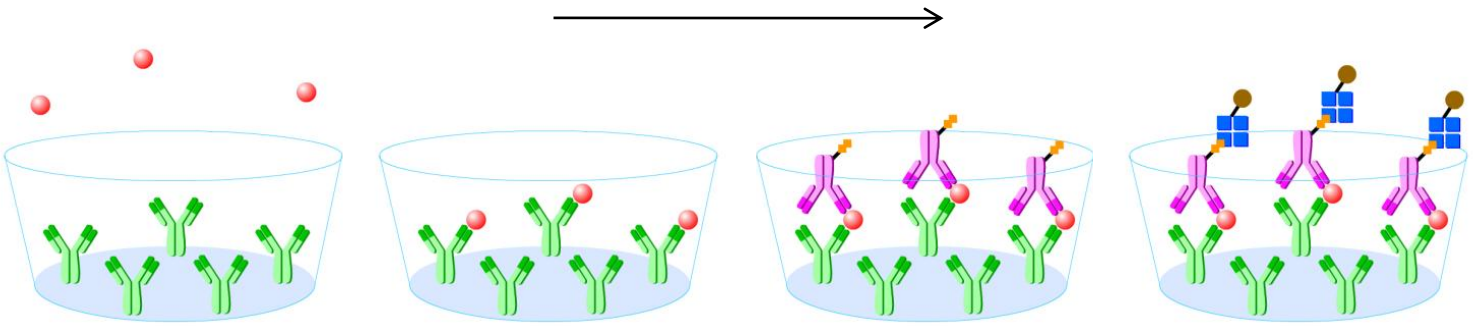
PRINCIPLE OF THE TEST

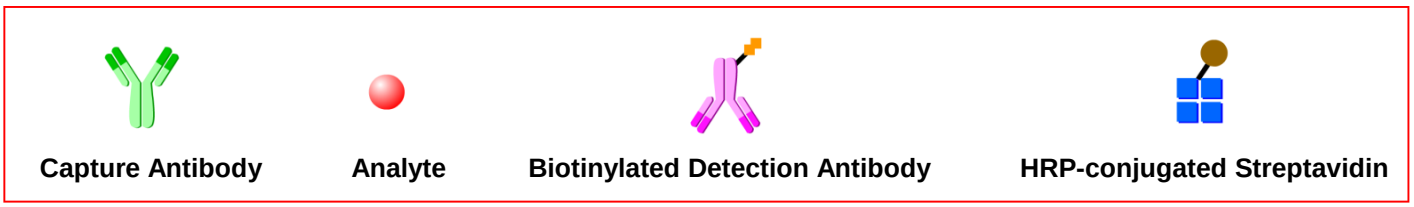
The Sino Biological ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for CD48 (BCM-1 / SLAMF2) coated on a 96-well plate. Standards and samples are added to the wells, and any CD48 (BCM-1 / SLAMF2) present binds to the immobilized antibody. The wells are washed and a biotinylated rabbit anti-CD48 polyclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". To produces color in proportion to the amount of CD48 (BCM-1 / SLAMF2) present in the sample strepavidin-HRP and TMB substrate solution are loaded. The absorbances of the microwell are read at 450 nm.

INTENDED USE

- The human CD48 (BCM-1 / SLAMF2) ELISA Pair Set is for the quantitative determination of human CD48.
- 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 - 0.4 mg/mL of mouse anti-CD48 monoclonal antibody. Dilute to a working concentration of 0.5 µg/mL in CBS before coating. (Catalog: # 10797-MM04)

Detection Antibody - 1.0 mg/mL of rabbit anti-CD48 polyclonal antibody. Dilute to a working concentration of 0.5 µg/mL in detection antibody dilution buffer before use.

Standard - Each vial contains 20 ng of recombinant CD48. Reconstitute standard powder with 1 mL detection antibody dilution buffer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 1000 pg/mL is recommended.

Streptavidin-HRP - 50 µL of streptavidin conjugated to horseradish-peroxidase. 1:2000 Dilution in detection antibody dilution buffer before use.

SOLUTIONS REQUIRED

CBS - 0.05M Na_2CO_3 , 0.05M $NaHCO_3$, 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 - 10 mg/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: Aliquot and store at -20℃ to -80℃ 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 -20℃ to -80℃ for up to 1 month. Avoid repeated freeze-thaw cycles.

Streptavidin-HRP: Store at 4℃ and protect it from prolonged exposure to light. **DO NOT FREEZE!** It is stable for up to 6 months from date of receipt.

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

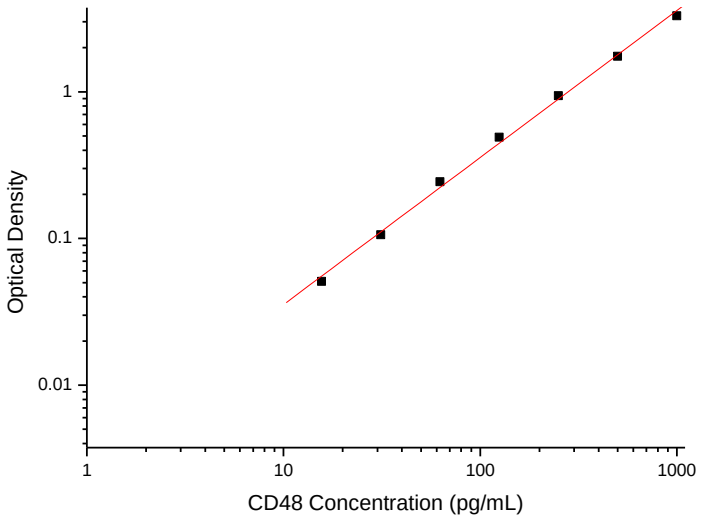
1. Add 100 μL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 μL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 100 μL of Streptavidin-HRP to each well. Incubate for 1 hour at room temperature.
6. Repeat the aspiration/wash as in step 2 of plate preparation.
7. 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.
8. Add 50 μL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
9. 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
15.625	0.051
31.25	0.106
62.5	0.244
125	0.490
250	0.939
500	1.746
1000	3.302

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of human CD48 (BCM-1 / SLAMF2) was determined to be approximately **15.625 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