DATA SHEET

Human CD36 / GP3B
ELISA Pair Set
(Platelet glycoprotein 4 / GPIV)
Catalog Number : SEK10752

BACKGROUND

Platelet glycoprotein4, also known as Leukocyte differentiation antygen CD36, GPIIIB, GP3B, FAT, and CD36, is integraf membrane protein fund on the surface of many cell types invertebrate animals. It belongs to the CD36 family and is found on platelets, erythrocytes, monocytes differentiated adipocytes, mammary epithelial cells, spleen cells and some skin microdermal endothelial cells. CD36 has also been implicated in hemostasis, thrombosis, malaria, inflammation, lipid metabolism and atherogenesis. CD36 seems to have numerous potential physiological functions. It binds to collagen, thrombospondin, anionic phospholipids and oxidized LDL. It directly mediates cytoadherence of Plasmodium falciparum parasitized erythrocytes and binds long chain fatty acids. CD36 may also function as a cell adhesion molecule and in the transport and/or as a regulator of fatty acid transport. Defects in CD36 are the cause of platelet glycoprotein IV deficiency also known as CD36 deficiency. As a receptor for thrombospondin1, CD36 plays a role in the regulation of angiogenesis, which may be a therapeutic strategy for controlling the dissemination of malignant neoplasms.

PRINCIPLE OF THE TEST

The Sino Biological ELISA Pair Set is a solid chase Sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specificfor CD36/GP3B coated on a 96-wellplate. Standards and samples are added to the wells, and any CD36/GP3B present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated mouseanti-CD36/GP3B monoclonal antibody is the nadded, producing an antibody-antigen antibody“sandwich”. The well sare again washed and TMB substrate solution is loaded, which produces color in proportion to the amount of CD36/GP3B present in the sample. To end the enzyme reaction, the stop solution is added and absorbances of the microwell are read at 450nm.

INTENDED USE

The human CD36/GP3B ELISA Pair Set is for the quantitative determination of human CD36. This ELISA Pair Set contains the Basic components required for the development of sandwich ELISAs.

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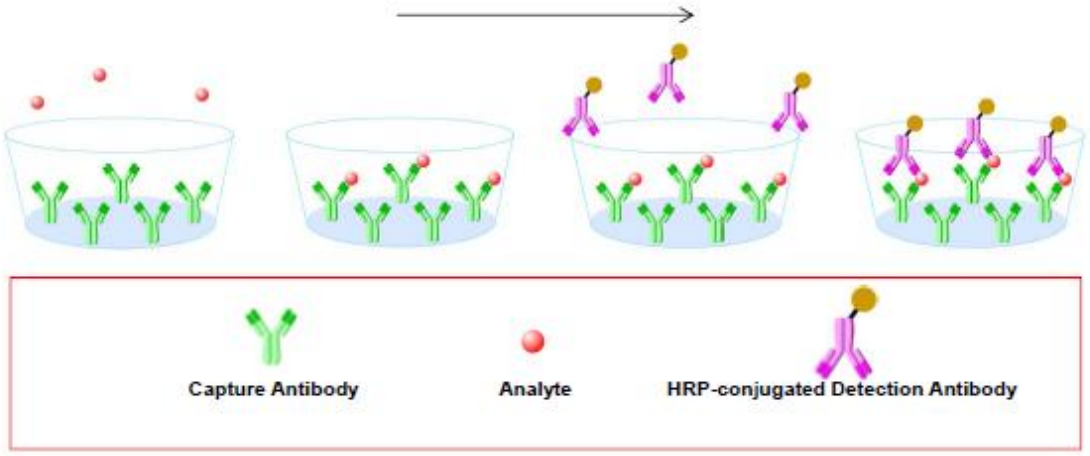
ASSAYPROCEDURESUMMARY



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

MATERIALSPROVIDED

Bring all reagents to room temperature before use.

Capture Antibody–0.5mg/mL of mouse anti CD36 monoclonal antibody. Dilute to a working concentration of 2.0µg/mL in CBS before coating.

DetectionAntibody–0.5mg/mL mouse anti-CD36 monoclonal antibody conjugated to horseradish-peroxidase (HRP). Dilute to working concentration of 2.0µg/mL in detection antibody dilution buffer before use.

Standard- Each vial contains 220ng of recombinant CD36. Reconstitute with 1mL detection antibody dilution buffer. After reconstitution, storeat -20℃to-70℃in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 20ng/mL is recommended.

SOLUTIONSREQUIRED

CBS -0.05M Na2CO3 , 0.05M NaHCO3 , pH 9.6, 0.2 µm filtered
TBS -25mM Tris, adjust pH to 7.4 by HCl
Wash Buffer -0.05% Tween 20 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
Substratestocksolution-10mg/ml TMB (Tetramethylbenzidine) in DMSO
Substratedilutionbuffer-0.05M Na2HPO4 and 0.025M citricacid; adjust pH to 5.5
Substrateworkingsolution- For each plate dilute 250µl substrate stock solution in 25 ml substrate dilution buffer and then add 80µl 0.75% H2O2, mix it well

Stop Solution -2 N H2SO4

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
Antibody should be protected from prolonged exposure to light. Aliquot the reagents and store at -20°Cto-70°Cin a manual defrost freezer.

GENERAL ELISA PROTOCOL

Plate Preparation

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 over night at 4°C.
Aspirate each well and wash with at least 300 µl 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.
Block plater by adding 300 µL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

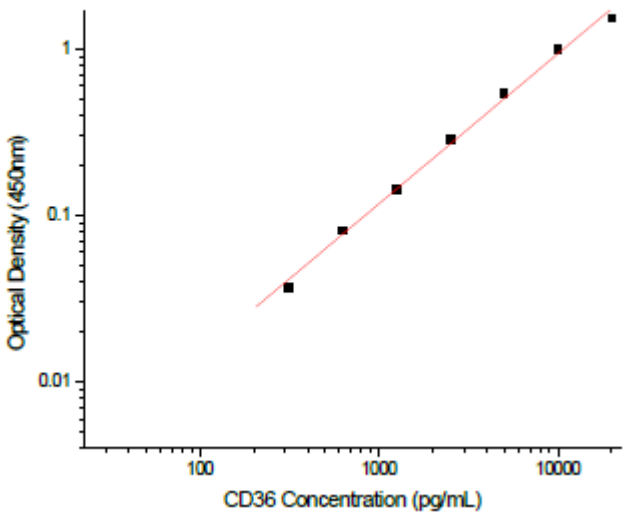
Assay Procedure
Add 100µL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature. Repeat the aspiration/wash as in step 2 of plate preparation. Add 100 µL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature. Repeat the aspiration/wash as in step 2 of plate preparation. Add 200µL 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. Add 50µL of stop solution to each well. Gently tap the plate to ensure thorough mixing. Determine the optical density of each well immediately, Rusing a microplate reader set to 450nm

CALCULATIONOFRESULTS

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 they-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 they-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.

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



Concentration (pg/ml)	Zero standard subtracted OD
0	0.000
312.5	0.037
625	0.081
1250	0.143
2500	0.286
5000	0.539
10000	0.994
20000	1.530

PERFORMANCE CHARACTERISTIC

SENSITIVITY
The minimum detectable dose of human CD36/GP3B was determined to be approximately **312.5pg/ml**. This is defined as at least three times standard deviations above the meanoptical 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 -70 ℃
	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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