Sino Biological
Biological Solution Specialist

Human CD55 / DAF ELISA Pair Set

Catalog Number : SEK10101
Lot Number: KW17OC2001

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

CD55, also well known as decay-accelerating factor (DAF), is a member of the RCA (regulators of complement activation) family characterized by four to 30 SCRs (short consensus repeats) in their plasma-exposed regions. It is a major regulator of the alternative and classical pathways of complement activation and is expressed on all serum-exposed cells. CD55 is physiologically acting as an inhibitor of the complement system, but is also broadly expressed in malignant tumours. DAF seems to exert different functions beyond its immunological role such as promotion of tumorigenesis, decrease of complement mediated tumor cell lysis, autocrine loops for cell rescue and evasion of apoptosis, neoangiogenesis, invasiveness, cell motility. It is commonly hijacked by invading pathogens, including many enteroviruses and uropathogenic Escherichia coli, to promote cellular attachment prior to infection. This 70-75 kDa glycoprotein CD55 containing four SCR modules is involved in the regulation of the complement cascade. It inhibits complement activation by suppressing the function of C3/C5 convertases, thereby limiting local generation or deposition of C3a/C5a and membrane attack complex (MAC or C5b-9) production. DAF has been identified as a ligand for an activation-associated, seven-transmembrane lymphocyte receptor, CD97, which is a receptor mediating attachment and infection of several viruses and bacteria. In addition, it has been shown that DAF regulates the interplay between complement and T cell immunity in vivo, and thus may be implicated in immune and tumor biology.

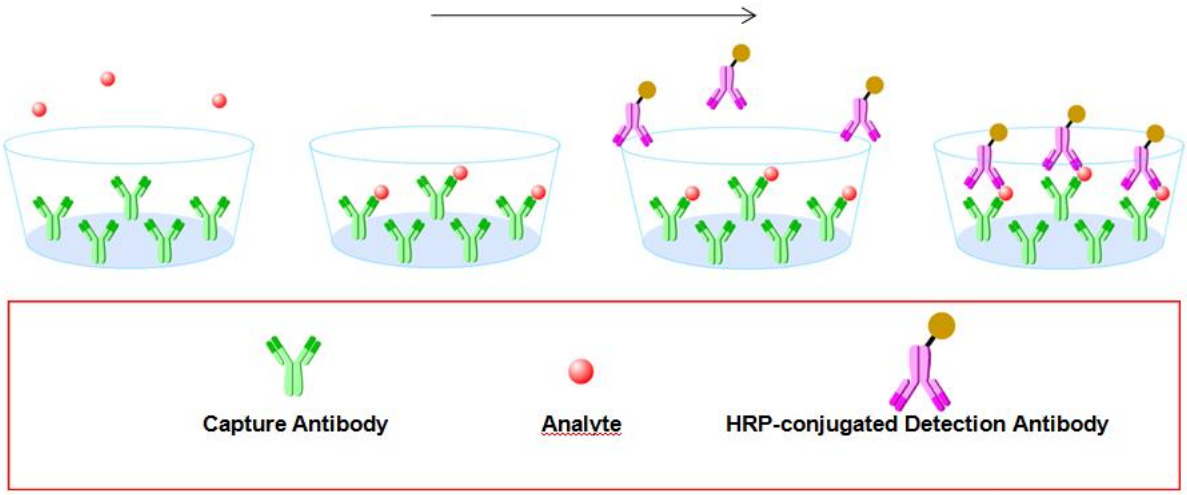
PRINCIPLE OF THE TEST

This ELISA Pair Set contains the basic components required for the development of sandwich ELISAs. The Sino Biological ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for CD55coated on a 96-well plate. Standards and samples are added to the wells, andany CD55 present binds to the immobilized antibody. The wells are washed and ahorseradish peroxidase conjugated mouse anti-CD55 monoclonal antibody is thenadded, producing an antibody-antigen-antibody "sandwich". The wells are againwashed and TMB substrate solution is loaded, which produces color in proportionto the amount of CD55 present in the sample. To end the enzyme reaction, thestop solution is added and absorbances of the microwell are read at 450 nm.

INTENDED USE

- The human CD55 ELISA Pair Set is for the quantitative determination of human CD55.
- 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 – 1.0 mg/mL of rabbit anti-CD55 monoclonal antibody (in PBS, pH 7.4). Dilute to a working concentration of 2 µg/mL in PBS before coating. (Catalog: # 10101-R028)

Detection Antibody – 0.2 mg/mL mouse anti-CD55 monoclonal antibody conjugated to horseradish-peroxidase (HRP) (in PBS, 50 % HRP-Protector, pH 7.4). Dilute to working concentration of 0.05 µg/mL in dilution buffer before use (Catalog: # 10101-MM03).

Standard – Each vial contains 290 ng of recombinant CD55. Reconstitute with 1 mL 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 dilution buffer, and a high standard of 8000 pg/mL is recommended.

Standard reconstitution tips: Add dilution buffer, gently mix it up and down 3~5 times. Avoid violent and long-time shock.

SOLUTIONS REQUIRED

PBS - 136.9 mM NaCl, 10.1 mM Na_2HPO_4, 2.7 mM KCl, 1.8 mM KH_2PO_4, pH 7.4, 0.2 µm filtered

Wash Buffer - 0.05% Tween20 in PBS, pH 7.2 - 7.4

Blocking Buffer - 2% BSA in Wash Buffer

Dilution Buffer - 0.1% 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: Store at 4℃ 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℃ 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.

ALTERNATIVE NAMES

CHAPLE, CR, CROM, DAF, TC

GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the capture antibody to the working concentration in PBS. 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

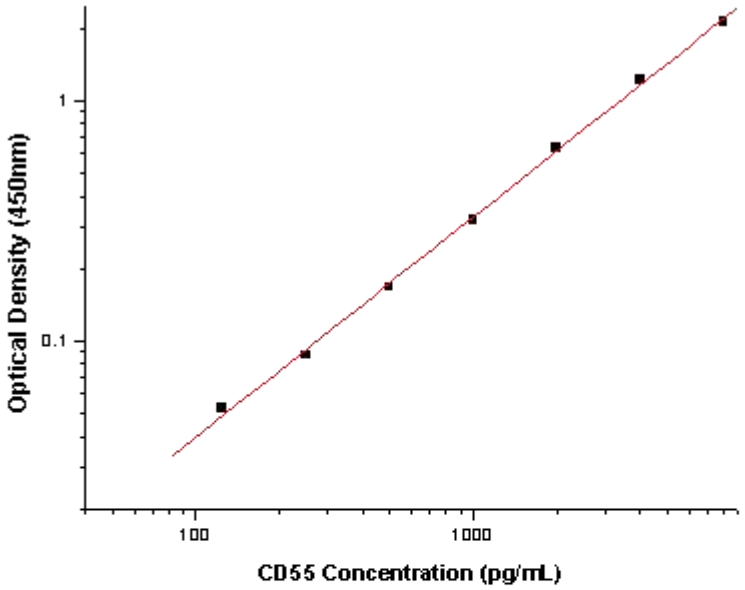
1. Add 100 μL of sample or standards in Dilution Buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 μL of the detection antibody, diluted in Dilution Buffer, to each well. Seal the plate and incubate 1 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
125	0.052
250	0.087
500	0.167
1000	0.317
2000	0.631
4000	1.225
8000	2.136

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of human CD55 was determined to be approximately 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.

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