Human Complement C1s ELISA Pair Set

Catalog Number : SEK10220

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

Complement is an integral component of the adaptive and innate immune systems and represents one of the major effector systems for the immune responses. The classical complement pathway is triggered by C1, a complex composed of the binding protein C1q and two proenzymes, C1r and C1s. Upon binding of IgG to the head of C1q, C1r undergoes autoactivation and in turn cleaves and activates C1s. C1r and C1s, the proteases responsible for activation and proteolytic activity of the C1 complex of complement, share similar overall structural organizations featuring five nonenzymic protein modules (two CUB modules surrounding a single EGF module, and a pair of CCP modules) followed by a serine protease domain. Besides highly specific proteolytic activities, both proteases exhibit interaction properties associated with their N-terminal regions. In contrast, C1r and C1s widely differ from each other by their glycosylation patterns: both proteins contain Asn-linked carbohydrates, but four glycosylation sites are present on C1r, and only two on C1s. As a highly specific serine protease, C1s executes the catalytic function of the C1 complex: the cleavage of C4 and C2, and thus instigates a sequence of activation steps of other components of the complement system, culminating in the formation of the membrane attack complex which induces cell lysis. Like other complement serine proteases C1s has restricted substrate specificity and it is engaged into specific interactions with other subcomponents of the complement system. The only other protein known to interact with C1s physiologically is SerpinC1, an inhibitor of serine protease, which inhibits C1s activity and thus plays a regulatory role in controlling the function of C1s enzyme.

PRINCIPLE OF THE TEST

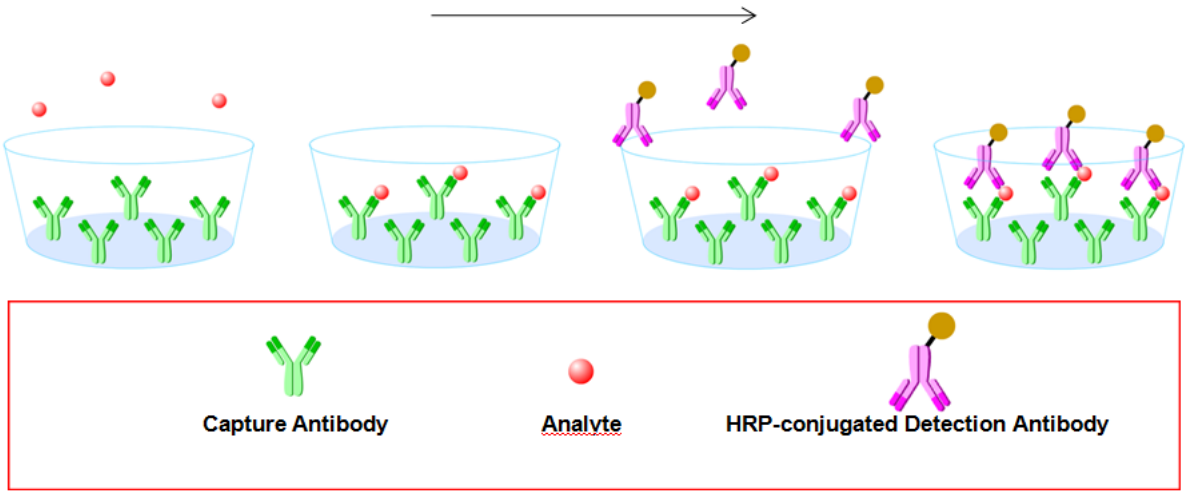
The Sino Biological ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for Human Complement C1s coated on a 96-well plate. Standards and samples are added to the wells, and any Human Complement C1s present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated mouse anti-Human Complement C1s 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 Human Complement C1s 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 Complement C1s ELISA Pair Set is for the quantitative determination of Human Complement C1s.

◆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 mg/mL of mouse anti-Human Complement C1s monoclonal antibody (in PBS, pH 7.4). Dilute to a working concentration of 2 μg/mL in PBS before coating. (Catalog: # 10220-MM02)

Detection Antibody – 0.2 mg/mL of mouse anti-Human Complement C1s monoclonal antibody conjugated to horseradish-peroxidase (HRP) (in PBS, 50 % HRP-Protector, pH 7.4, store at 4℃). Dilute to working concentration of 0.15 μg/mL in detection antibody dilution buffer before use. (Catalog: # 10220-MM04)

Standard – Each vial contains 180 ng of recombinant Human Complement C1s. Reconstitute with 1 mL detection antibody dilution buffer. After reconstitution, store at -20℃ to -80℃ in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 6000 pg/mL is recommended.

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

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: 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.

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

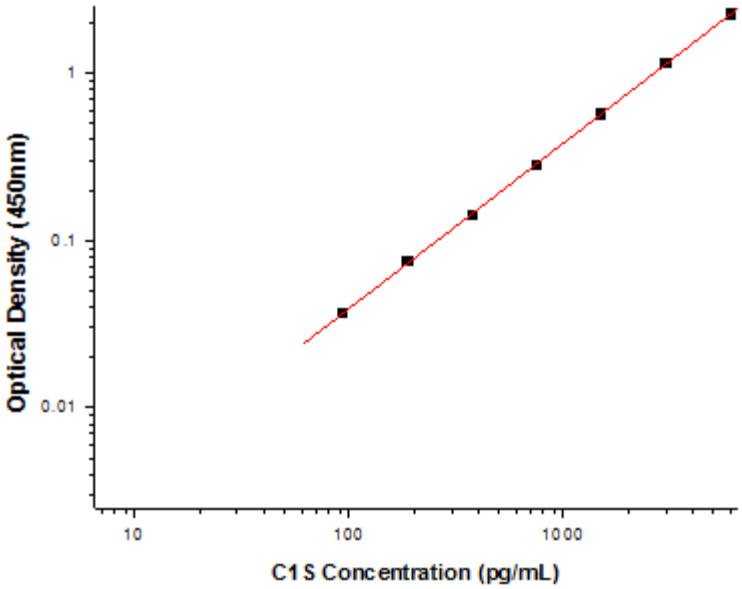
1.Add 100 μL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2.Repeat the aspiration/wash as in step 2 of plate preparation.
3.Add 100 μL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and 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
93.75	0.037
187.5	0.075
375	0.142
750	0.281
1500	0.568
3000	1.157
6000	2.256

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of Human Complement C1s was determined to be approximately **93.75 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