



Sino Biological  
Biological Solution Specialist

---

# Human C5a ELISA Pair Set

**Catalog Number : SEKA10604**

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

C5a is a protein fragment released from complement component C5. This 74 amino acid peptide in humans is generated by the cleavage of C5a convertase on the C5  $\alpha$ -chain during the classical, alternative, and lectin pathways of complement activation. The structure of C5a includes a core region consisting of four, anti-parallel  $\alpha$ -helices held together by three disulfide linkages and a structured C-terminal tail, and C5a is rapidly metabolised by carboxypeptidase B to a 73 amino acid low activity form, C5a des-Arg. C5a is an extremely potent proinflammatory mediator, as well as a potent chemotactic factor for neutrophils and other leukocytes. It causes histamine release, increases in vascular permeability, induces several cytokines production from leukocytes, enhances neutrophil-endothelial cell adhesion, and augments the humoral and cell-mediated immune response. C5a is quickly metabolised by carboxypeptidases, forming the less potent C5adesArg. Acting via a classical G protein-coupled receptor, CD88, C5a and C5adesArg exert a number of effects essential to the innate immune response, while their actions at the more recently discovered non-G protein-coupled receptor, C5L2 (or GPR77), remain unclear. The widespread expression of C5a receptors throughout the body allows C5a to elicit a broad range of effects. Thus, C5a has been found to be a significant pathogenic driver in a number of immunoinflammatory diseases, making C5a inhibition an attractive therapeutic strategy. C5a is a strong chemoattractant and is involved in the recruitment of inflammatory cells such as neutrophils, eosinophils, monocytes, and T lymphocytes, in activation of phagocytic cells and release of granule-based enzymes and generation of oxidants, all of which may contribute to innate immune functions or tissue damage. Accordingly, the anaphylatoxin C5a is implicated in a variety of diseases such as rheumatoid arthritis, systemic lupus erythematosus, reperfusion injury, Alzheimer's disease, and sepsis.

## 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 C5a coated on a 96-well plate. Standards and samples are added to the wells, and any Human C5a present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated mouse anti-Human C5a 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 C5a 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 C5a ELISA Pair Set is for the quantitative determination of Human C5a.
- ◆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 C5a monoclonal antibody (in PBS, pH 7.4). Dilute to a working concentration of 2 µg/mL in PBS before coating. (Catalog: # 10604-MM08)

**Detection Antibody** – 0.2 mg/mL of mouse anti-Human C5a monoclonal antibody conjugated to horseradish-peroxidase (HRP) (in PBS, 50 % HRP-Protector, pH 7.4, store at 4°C). Dilute to working concentration of 0.5 µg/mL in detection antibody dilution buffer before use. (Catalog: # 10604-MM32)

**Standard** – Each vial contains 18 ng of recombinant Human C5a. Reconstitute with 1 mL detection antibody dilution buffer. After reconstitution, store at -20°C to -80°C in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 1000 pg/mL is recommended.

## SOLUTIONS REQUIRED

**PBS** - 136.9 mM NaCl, 10.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 2.7 mM KCl, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4, 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<sub>2</sub>HPO<sub>4</sub> 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<sub>2</sub>O<sub>2</sub> , mix it well

**Stop Solution** - 2 N H<sub>2</sub>SO<sub>4</sub>

## 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  $-20^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$  for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

**Detection Antibody:** Store at  $4^{\circ}\text{C}$  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  $-20^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$  for up to 6 months from date of receipt. Aliquot and store the reconstituted standard at  $-80^{\circ}\text{C}$  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°C.
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

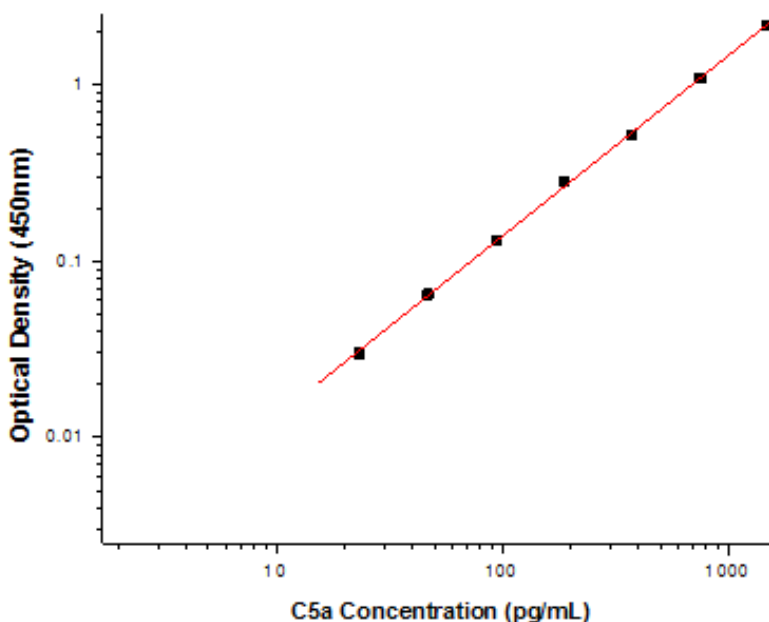
1. Add 100 µL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 µL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate 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
23.44	0.030
46.88	0.065
93.75	0.131
187.5	0.281
375	0.521
750	1.090
1500	2.170

## PERFORMANCE CHARACTERISTIC

### SENSITIVITY

The minimum detectable dose of Human C5a was determined to be approximately **23.44 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
<b>No signal</b>	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
<b>Poor Standard Curve</b>	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 °C
	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
<b>Poor detection value</b>	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
<b>High Background</b>	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
<b>Non-specificity</b>	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												

# **Human C5a ELISA Pair Set**

## **Notes**