C5a ELISA Kit (Catalog# TBS3001)

DESCRIPTION

Complement component C5a is a multifunctional proinflammatory mediator produced by complement cascade activation and causes inflammatory responses and cytokine production in septic shock, allergy, trauma, ischemic heart disease, pain, neurodegenerative diseases, and other several autoimmune diseases. C5a is rapidly cleaved to the C5a-DesArg form by the endogenous serum carboxypeptidase enzyme.

The C5a ELISA Kit can be used for *in vitro* determination of mouse C5a in different tissue samples including skin, muscle, nervous system and other biological samples. The microplate is coated with murine C5a monoclonal antibody. C5a present in the sample or standard binds to the antibodies absorbed to the microwell. A biotin-conjugated anti-murine C5a antibody is then added and binds to murine C5a captured by the first antibody. After adding streptavidin-horseradish peroxidase (HRP) an antibody-antigen-antibody "sandwich" is produced. Finally, substrate solution reactive with HRP is added to the well, exhibiting a blue color in direct proportion to the amount of C5a in the initial sample. The reaction is terminated by the addition of an acid, and absorbance is measured at 450nm. The concentration of C5a in the samples is determined by comparing the O.D. of the samples to the standard curve.

APPLICATIONS

Direct Assays: C5a concentrations in different tissues.

KIT CONTENTS

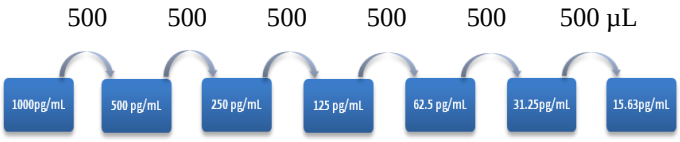
Reagents	Quantity
Precoated 96-well Plate	1
Standard	2x 100µL
Detection A	1x 100µL
Detection B	1x 100µL
Sample Diluent (10x concentrate)	1x 12ml
TMB Substrate Solution	1x 12ml
Stop Solution	1x 12ml
Wash Solution (20x concentrate)	1x 50ml
Plate Sealers	4

Storage conditions: Store the kit at 4°C and standard at -20°C, respectively.
Shelf life: 12 months after receipt.

PROCEDURES

Reagent Preparation:

1. Bring all reagents to room temperature (18-25˚C) before use.
2. Wash Buffer: Add 50mL of 20x concentrated Wash Buffer into 950mL distilled water and mix thoroughly.
3. Reagent Diluent: Dilute 10mL of 10x concentrated Sample Diluent into 90mL of deionized water (1:10) before use.
4. Dilute Detection A: Add 100µL of the Detection A into 10 mL of the Reagent Diluents for a 96-well plate.
5. Dilute Detection B: Add 100µL into 10mL of the Reagent Diluent for a 96-well plate.
6. Standard: Set up 7 points of C5a standard concentrations: 1000, 500, 250, 125, 62.5, 31.25 and 15.63 pg/mL as shown in the diagram below.
7. Label 7 tubes of 1.5ml from 1-7. Add 900 µL of the Reagent Diluents to the tube #1. Add 500 µL of the Reagent Diluents to tubes #2-7.
8. Add 100µL of the C5a standard into tube #1 and vortex. This is Standard tube #1 with a concentration of 1000 pg/mL.
9. Standards #2-7 are then prepared by performing a 1:2 dilution of the preceding standard. Mix each tube thoroughly before the next transfer. For example, to make Standard #2, remove 500 µL of Standard #1 and add it to tube #2 and vortex and so on. The Reagent Diluents serves as the zero standard (0 pg/ml).



Test Procedures

1. Add 100µL of standards, blank, and sample into plate in duplicate manner. Seal the plate and incubate for 2 hrs at room temperature.
2. Remove sealer and empty wells. Wash the plate 3 times with 150µL Wash Buffer.
3. Add 100µL of the diluted Detection A to each well. Seal the plate and incubate for 2 hrs at room temperature.
4. Repeat washing step 2.

5. Add 100µL of the diluted Detection B to each well. Seal the plate and incubate for 20 minutes at RT.

6. Repeat washing Step 2.

7. Add 100µL of Substrate Solution to each well. Incubate for 20 minutes at RT. Protect from light.

8. Add 50µL of Stop Solution to each well when the highest standard has developed a dark blue color. Gently tap the plate to ensure thoroughly mixing.

9. Determine the optical density of each well immediately, using a microplate reader set to 450nm.

CALCULATION of Result

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, 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. The data may be linearized by plotting the log of the mouse C5a concentrations versus the log of the O.D. and the best-fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

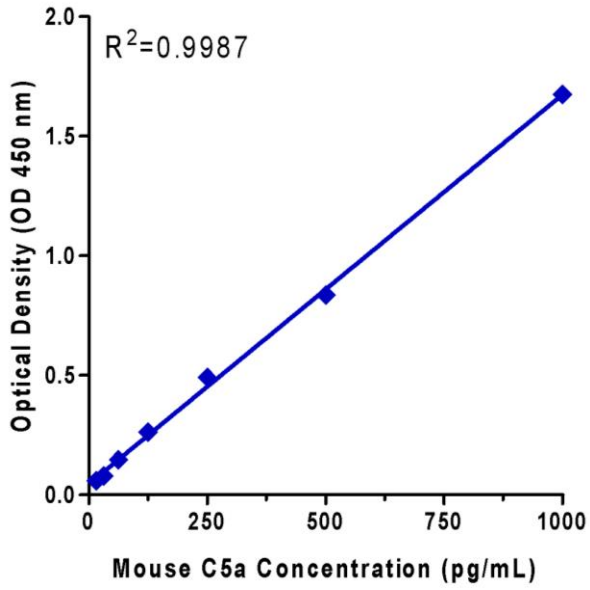
- Mircorplate reader
- Pipetttes and Pipette tips
- Microtubes

MAIN FEATURES

Sensitivity: The minimum detectable dose of mouse C5a is typically less than 7.8 pg/mL.

Specificity: This assay recognizes recombinant and natural mouse C5a. No significant cross-reactivity or interference is observed.

Standard Curve of C5a



REFERENCES

1. Huber-lang M. et al. Generation of C5a in the absence of C3: a new complement activation pathway. *Nat. Med. 12:* 682-687(2006).

2. Leihase I. et al. Inhibition of the alternative complement activation pathway in traumatic brain injury by a monoclonal anti-factor B antibody: a randomized placebo-controlled study in mice. JNI. *4:* 13-20 (2007).

RELATED PRODUCTS:

BSA Standard solution (catalog# TBS5002)

Protein Cell Lysis Buffer (catalog# TBS5001)

Protein Assay Kit (Catalog# TBS2005)

Mouse C3a ELISA (Catalog# TBS3020)

TMB Substrate System (Catalog# TBS5021)