

**Mouse C5a ELISA Kit (Mouse Plasma/Serum C5a) Cat. #: T301****Storage & Ship:** Store condition, see table below. Ship at 4°C.**Shelf Life:** 12 months**Product Size:** 12 x 8 well strips (1 x 96-well plate)**Product Description** (This product is for research use only.)

The C5a ELISA Kit is for the 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 microwells. Then, a biotin-conjugated anti-murine C5a antibody is added and binds to murine C5a captured by the first antibody. Next, streptavidin-horseradish peroxidase (HRP) is added, producing an antibody-antigen-antibody "sandwich". Finally, substrate solution reactive with HRP is added to the well, and exhibits 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 450 nm. 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 COMPONENTS	Quantity	Store at	Ship at
Precoated 96-well Plate	1	4°C	4°C
Standard	2 x 100 µL	-20°C	4°C
Detection A	1 x 100 µL	-20°C	4°C
Detection B	1 x 100 µL	-20°C	4°C
Sample Diluent ( 10 x concentrate)	1 x 12 ml	-20°C	4°C
TMB Substrate Solution	1 x 12 ml	4°C	4°C
Stop Solution	1 x 12 ml	4°C	RT or 4°C
Wash Solution (20 x concentrate)	1 x 50 ml	4°C	RT or 4°C
Plate Sealers	4	4°C	RT or 4°C

**Reagent Preparation:**

1. Bring all reagents to room temperature (18-25°C) before use.
2. Wash Buffer: Add 50 mL of 20x concentrated Wash Buffer into 950 mL distilled water and mix thoroughly.
3. Reagent Diluent: Dilute 10 mL of 10x concentrated Sample Diluent into 90 mL of deionized water ( 1:10 ) before use.

4. Dilute Detection A: Add 100  $\mu\text{L}$  of the Detection A into 10 mL of the Reagent Diluents for 96-well plate.
5. Dilute Detection B: Add 100  $\mu\text{L}$  into 10mL of the Reagent Diluent for 96-well plate.
6. Standard: Set up 7 points of C5a standard concentrations: 1000, 500, 250, 125, 62.5, 31.25 and 15.63pg/mL as shown in the diagram below.
7. Label 7 tubes of 1.5ml from 1-7. Add 900  $\mu\text{L}$  of the Reagent Diluents to the tube #1. Add 500  $\mu\text{L}$  of the Reagent Diluents to tubes #2-7.
8. Add 100  $\mu\text{L}$  of the C5a standard into tube #1 and vortex. This is Standard tube #1 with a concentration of 1000pg/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  $\mu\text{L}$  of Standard #1 and add it to tube #2 and vortex and so on. The Reagent Diluent serves as the zero standard (0 pg/ml).

500    500    500    500    500    500  $\mu\text{L}$



## Protocol

1. Add 100  $\mu\text{L}$  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 250 $\mu\text{L}$  Wash Buffer.
3. Add 100  $\mu\text{L}$  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 100 $\mu\text{L}$  of the diluted Detection B to each well. Seal the plate and incubate for 20 min at RT.
6. Repeat washing Step 2.
7. Add 100  $\mu\text{L}$  of Substrate Solution to each well. Incubate for 20 min at RT. Protect from light.
8. Add 50  $\mu\text{L}$  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.

## MATERIALS REQUIRED, BUT NOT PROVIDED

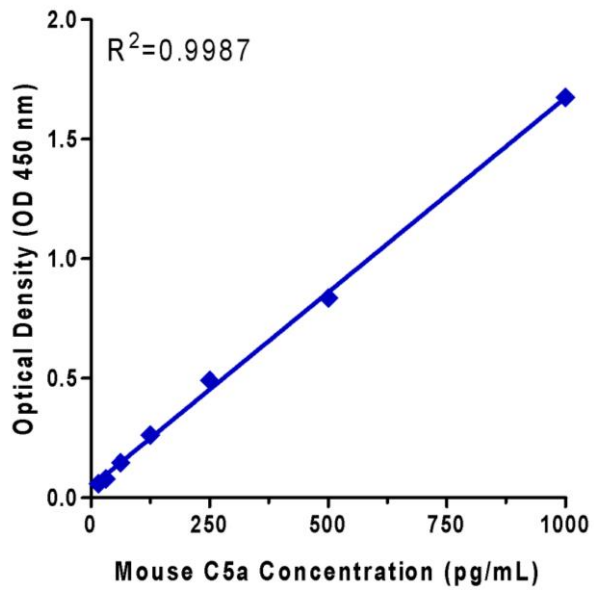
- Microplate reader
- Pipettes and Pipette tips
- Microtubes

## KEY 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



### RELATED PRODUCTS:

BSA Standard solution (catalog# T5002)

Protein Cell Lysis Buffer (catalog# T5001)

Protein Assay Kit (Catalog# T2005)

Mouse C3a ELISA (Catalog# T3020)

TMB Substrate System (Catalog# T5021)