

Datasheet for ABIN6962855

C5A ELISA Kit[1 Image](#)[1 Publication](#)[Go to Product page](#)

Overview

Quantity:	96 tests
Target:	C5A (C5a)
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Detection Range:	62.5 pg/mL - 4000 pg/mL
Minimum Detection Limit:	62.5 pg/mL
Application:	ELISA

Product Details

Purpose:	The kit is a sandwich enzyme immunoassay technique for the in vitro quantitative measurement in various sample types.
Sample Type:	Cell Culture Supernatant, Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This kit recognizes Mouse C5a in samples. No Significant cross-reactivity or interference between Mouse C5a and analogues was observed.
Sensitivity:	37.5 pg/mL
Components:	<ul style="list-style-type: none">• Pre-coated, ready to use 96-well strip plate, flat bottom• Plate sealer for 96 wells• Reference Standard• Reference Standard & Sample Diluent

Product Details

- Biotinylated Detection Antibody (100 x concentrate)
- HRP Conjugate (100 x concentrate)
- Biotinylated Detection Antibody Diluent
- HRP Conjugate Diluent
- Substrate Reagent
- Stop Solution
- Wash Buffer (25 x concentrate)
- Instruction manual

Target Details

Target: C5A (C5a)

Alternative Name: Complement Component 5a ([C5a Products](#))

Background: C5a

Pathways: [Complement System](#), [Carbohydrate Homeostasis](#)

Application Details

Sample Volume: 100 μ L

Assay Time: 3.5 h

Plate: Pre-coated

Protocol:

1. Add 100 μ L standard or sample to each well. Incubate for 90 min at 37 °C.
2. Remove the liquid. Add 100 μ L Biotinylated Detection Antibody. Incubate for 1 hour at 37 °C.
3. Aspirate and wash 3 times.
4. Add 100 μ L HRP Conjugate. Incubate for 30 min at 37 °C.
5. Aspirate and wash 5 times.
6. Add 90 μ L Substrate Reagent. Incubate for 15 min at 37 °C.
7. Add 50 μ L Stop Solution. Read at 450 nm immediately.
8. Calculation of results.

Reagent Preparation:

1. Bring all reagents to room temperature (18~25 °C) before use. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.
2. Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40 °C water bath and mix it gently until the crystals have completely dissolved
3. Standard working solution: Centrifuge the standard at 10,000xg for 1 min. Add 1.0 mL of Reference Standard & Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 4000 pg/mL. Then make serial dilutions as needed. The recommended

Application Details

dilution gradient is as follows: 4000, 2000, 1000, 500, 250, 125, 62.50, 0 pg/mL. Dilution method: Take 7 EP tubes, add 500 µL of Reference Standard & Sample Diluent to each tube. Pipette 500 µL of the 4000 pg/mL working solution to the first tube and mix up to produce a 2000 pg/mL working solution. Pipette 500 µL of the solution from the former tube into the latter one according to these steps. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.

4. Biotinylated Detection Antibody working solution: Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100x Concentrated Biotinylated Detection Antibody to 1x working solution with Biotinylated Detection Antibody Diluent.
5. Concentrated HRP Conjugate working solution: Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100x Concentrated HRP Conjugate to 1x working solution with Concentrated HRP Conjugate Diluent.

Sample Preparation:

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturation may occur in these samples, leading to false results. Samples should therefore be stored for a short period at 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thaw cycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determine compatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance. The recommended dilution factor is for reference only.
- Please estimate the concentration of the samples before performing the test. If the values are not in the range of the standard curve, the optimal sample dilution for the particular experiment has to be determined.

Assay Precision:

Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level Mouse C5a were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level Mouse C5a were tested on 3 different plates, 20 replicates in each plate.

Both intra-CV and inter-CV are < 10 %.

Restrictions:

For Research Use only

Handling

Storage:

4 °C, -20 °C

Storage Comment:

1. For unopened kit: All reagents should be stored according to the labels on the vials, so they are stable up to 12 months after receipt of the kit. The Reference Standard, Biotinylated

Handling

Detection Antibody, HRP Conjugate and the 96-well stripe plate should be stored at -20 °C upon receipt while the other reagents should be stored at 4 °C.

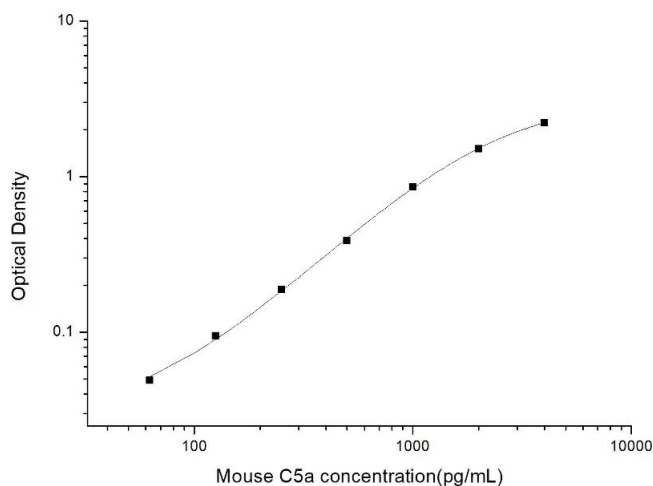
2. For used kit: When the kit is used, the remaining reagents need to be stored according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and zip-seal the foil pouch.

Expiry Date: 12 months

Publications

Product cited in: D'Angelo, Mangini, Fonderico, Fulle, Mayo, Aramini, Marigiò: "Inhibition of osteoclast activity by complement regulation with DF3016A, a novel small-molecular-weight C5aR inhibitor." in: **Biomedicine & pharmacotherapy**, Vol. 123, pp. 109764, (2020) ([PubMed](#)).

Images



ELISA

Image 1. Typical standard curve