



Datasheet for ABIN6955017

C5A ELISA Kit



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Overview

Quantity:	96 tests
Target:	C5A (C5a)
Reactivity:	Rat
Method Type:	Sandwich ELISA
Detection Range:	0.156 ng/mL - 10 ng/mL
Minimum Detection Limit:	0.156 ng/mL
Application:	ELISA

Product Details

Purpose:	The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of C5a in rat serum, plasma.
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of Complement Component 5a (C5a)
Sensitivity:	0.061 ng/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• Standard Diluent

Product Details

- Detection Reagent A
- Detection Reagent B
- Assay Diluent A
- Assay Diluent B
- Reagent Diluent (if Detection Reagent is lyophilized)
- TMB Substrate
- Stop Solution
- Wash Buffer (30 x concentrate)
- Instruction manual

Target Details

Target: C5A (C5a)

Abstract: [C5a Products](#)

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

Application Details

Comment: Information on standard material:
The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative.

Information on reagents:

The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them.

Information on antibodies:

The provided antibodies and their host vary in different kits.

Sample Volume: 100 µL

Assay Time: 3 h

Plate: Pre-coated

Protocol:

1. Prepare all reagents, samples and standards,
2. Add 100µL standard or sample to each well. Incubate 1 hours at 37 °C,
3. Aspirate and add 100µL prepared Detection Reagent A. Incubate 1 hour at 37 °C,

4. Aspirate and wash 3 times,
5. Add 100µL prepared Detection Reagent B. Incubate 30 minutes at 37 °C,
6. Aspirate and wash 5 times,
7. Add 90µL Substrate Solution. Incubate 10-20 minutes at 37 °C,
8. Add 50µL Stop Solution. Read at 450nm immediately.

Reagent Preparation:

1. Bring all kit components and samples to room temperature (18-25 °C) before use.
2. Standard - Reconstitute the Standard with 0.5mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 10ng/mL. Prepare 7 tubes containing 0.25mL Standard Diluent and produce a double dilution series. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.312ng/mL, 0.156ng/mL, and the last tube with Standard Diluent is the blank as 0ng/mL.
3. Detection Reagent A and Detection Reagent B - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute to the working concentration with Assay Diluent A and B, respectively (1:100).
4. Wash Solution - Dilute 20 mL of Wash Solution concentrate (30x) with 580 mL of deionized or distilled water to prepare 600 mL of Wash Solution (1x).
5. TMB substrate - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Note:

1. Making serial dilution in the wells directly is not permitted.
2. Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37 °C directly.
3. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL for once pipetting.
4. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.
5. If crystals have formed in the Wash Solution concentrate (30x), warm to room temperature and mix gently until the crystals are completely dissolved.
6. Contaminated water or container for reagent preparation will influence the detection result.

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

Application Details

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. Samples should then be diluted with PBS (pH =7.0-7.2).

Assay Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level of target were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level of target were tested on 3 different plates, 8 replicates in each plate. CV(%) = SD/meanX100 Intra-Assay: CV < 10% Inter-Assay: CV < 12%
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Restrictions:	For Research Use only
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Handling

Precaution of Use:	The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
Storage:	4 °C/-20 °C
Storage Comment:	1. For unopened kit: All reagents should be stored according to the labels on the vials. The Standard, Detection Reagent A, Detection Reagent B, and 96-well Strip Plate should be stored at -20 °C upon receipt, while the other reagents should be stored at 4 °C. 2. For opened kits: the remaining reagents must be stored according to the above storage conditions. In addition, please return the unused wells to the foil pouch containing the desiccant and seal the foil pouch with the zipper.
Expiry Date:	6 months

Publications

Product cited in:	Zuo, Yu, Guo, Wang, Qian, Yi, Lu, Lv, Subjeck, Zhou, Sanyal, Chen, Wang: "Scavenger receptor A restrains T-cell activation and protects against concanavalin A-induced hepatic injury." in: Hepatology (Baltimore, Md.) , Vol. 57, Issue 1, pp. 228-38, (2013) (PubMed).
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ELISA

Image 1. Typical standard curve

