APA388Hu61 100μg
Active Complement Component 5a (C5a)
Organism Species: Homo sapiens (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression.
Host: 293F cell
Residues: Thr678~Arg751
Tags: N-terminal His Tag and C-terminal Fc Region of Human IgG1
Purity: >95%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 5% Trehalose .
Applications: Cell culture; Activity Assays; In vivo assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 8.9
Predicted Molecular Mass: 35.0kDa
Accurate Molecular Mass: 44kDa as determined by SDS-PAGE reducing conditions.
Phenomenon explanation:
The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not

vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

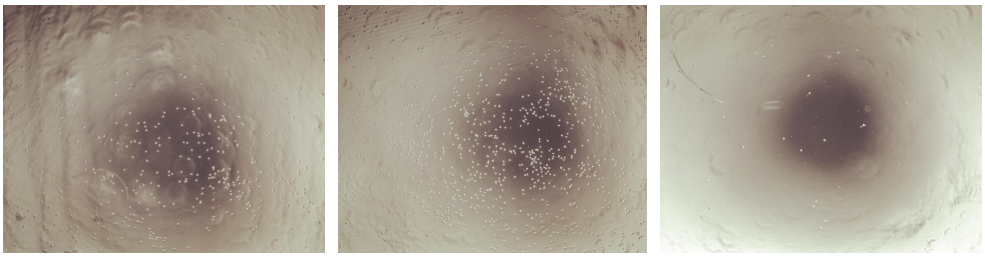
Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed.The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

TLQ KKIEEIAAKY KHSVVKKCCY DGACVNNDET CEQRAARISL GPRCIKAFTE CCVVASQLRA NISHKDMQLG R

[ACTIVITY]

Complement Component 5a (C5a) is a component of the complement system which plays a key role in promoting migration and adherence of neutrophils and monocytes to vessel walls. C5a has been proven to be able to induce chemotactic migration of THP-1 cells. Therefore, chemotaxis assay used 24-well microchemotaxis system was undertaken to detect the chemotactic effect of C5a on the human monocytic cell line THP-1. Briefly, THP-1 cells were seeded into the upper chambers (100μL cell suspension, 10^6 cells/mL in RPMI 1640 with 0.5% FBS) and C5a (50ng/mL and 100ng/mL diluted separately in serum free RPMI 1640) was added in lower chamber with a polycarbonate filter (8μm pore size) used to separate the two compartments. After incubation at 37°C with 5% CO_2 for 2h, the filter was removed, then cells in low chamber were observed by inverted microscope at low magnification (×100) and the number of migrated cells were counted at high magnification (×400) randomly (five fields for each filter). Result: C5a is able to induce migration of THP-1 cells. The migrated THP-1 cells in low chamber at low magnification (×100) were shown in Figure 1. Five fields of each chamber were randomly chosen to count the migrated cells at high magnification (×400) and the statistical data was shown in Figure 2.



A B C

Figure 1. The chemotactic effect of C5a on THP-1 cells.

(A) THP-1 cells were seeded into the upper chambers and 50ng/mL C5a was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation for 3h;

(B) THP-1 cells were seeded into the upper chambers and 100ng/mL C5a was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation for 3h;

(C) THP-1 cells were seeded into the upper chambers and serum free RPMI 1640 without C5a was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation for 3h.

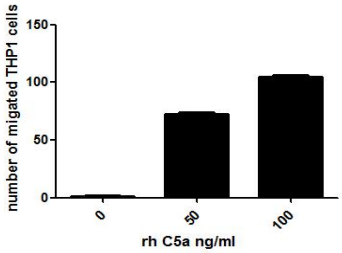


Figure 2. The chemotactic effect of C5a on THP-1 cells.

The activity of recombinant human C5a was also measured by its ability to induce N-acetyl- β -D-glucosaminidase release from differentiated U937 human histiocytic lymphoma cells. 3.2*106 differentiated U937 cells were added to the 24-well plate and different concentrations of rhC5a was added to the 24-well plate and incubated at 37 ℃ for 3min. The cells were centrifuged at 400 g for 3min, and the supernatant contained N-acetyl- β -D-glucosaminidase. The enzyme activity of N-acetyl- β -D-glucosaminidase was measured by the substrate of 4-Nitrophenyl 2-acetamido-2-deoxy- β -D-glucopyranoside. The result was shown in figure 1, It was obvious that rhC5a can significantly induce N-acetyl- β

-D-glucosaminidase release.

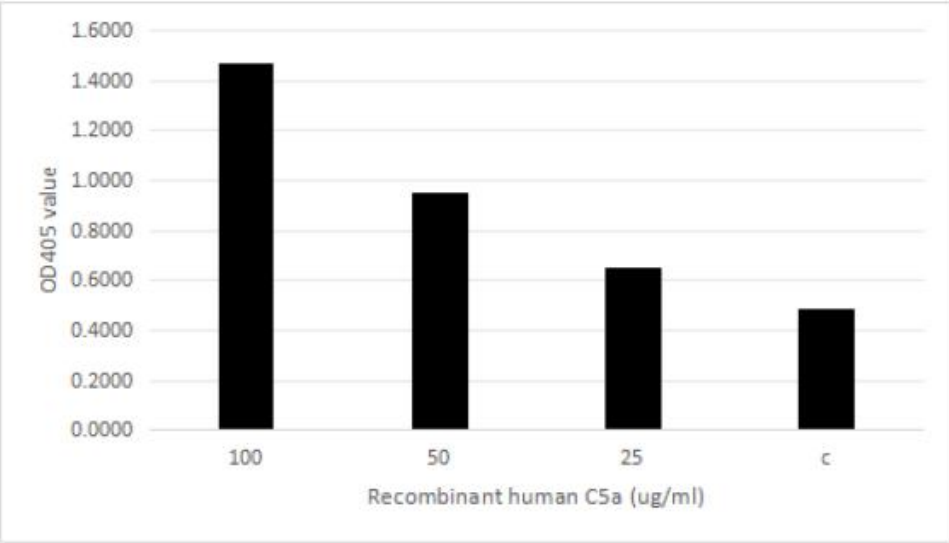


Figure 3. The activity of rhC5a on differentiated U937 cells

[IDENTIFICATION]

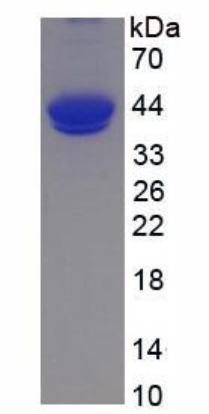


Figure 4. SDS-PAGE

Sample: Active recombinant C5a, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.