APA388Hu61 50μg
Active Complement Component 5a (C5a)
Organism Species: Homo sapiens (Human)
Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Thr678~Arg751

Tags: Two Tags, His-tag and Fc-tag

Purity: >95%

Buffer Formulation: 10mM 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.

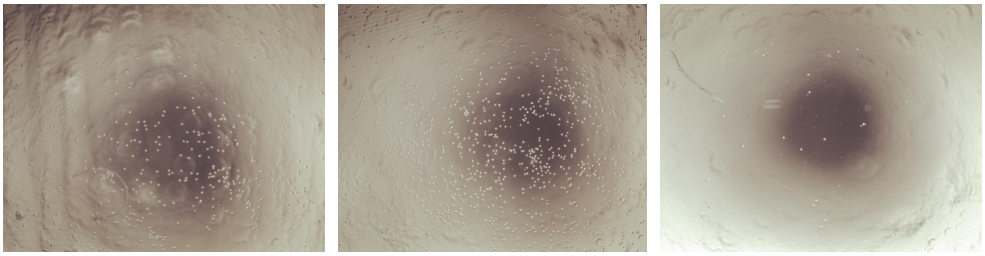
Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed.The loss rate is less than 5% within the expiration date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

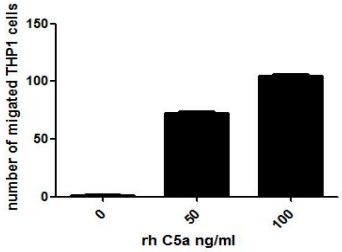


Figure 2. The chemotactic effect of C5a on THP-1 cells.

[IDENTIFICATION]

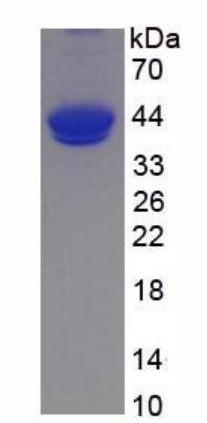


Figure 3. SDS-PAGE

Sample: Active recombinant C5a, Human

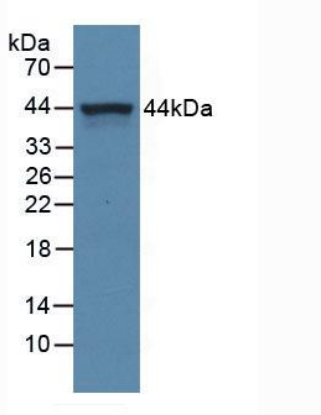


Figure 4. Western Blot

Sample: Recombinant C5a, Human;

Antibody: Rabbit Anti-Human C5a Ab (PAA388Hu06)

[IMPORTANT NOTE]

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