APA388Mu62 100µg Active Complement Component 5a (C5a) Organism Species: *Mus musculus (Mouse) 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: Asn679~Arg755

Tags: N-terminal His Tag and C-terminal Fc Region of Human IgG1

Purity: >90%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: PBS, pH7.4, containing 5% Trehalose .

Original Concentration: 200µg/mL

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 9.4

Predicted Molecular Mass: 39.9kDa

Accurate Molecular Mass: 39&40kDa 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.

[<u>USAGE</u>]

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]

 $\label{eq:linear} NLHLLRQKIEEQAAKYKHSVPKKCCYDGARVNFYETCEERVARVTIGPLCIRAFNECCTIANKIRKESPHKPVQLGR$

[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. 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 $^{\circ}$ C 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 1. The activity of rmC5a on differentiated U937 cells

[IDENTIFICATION]



Figure 2. Gene Sequencing (extract)

kDa 70
44
33
26
22
18
14
10

Figure 3. SDS-PAGE

Sample: Active recombinant C5a, Mouse

[<u>IMPORTANT NOTE</u>]

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.