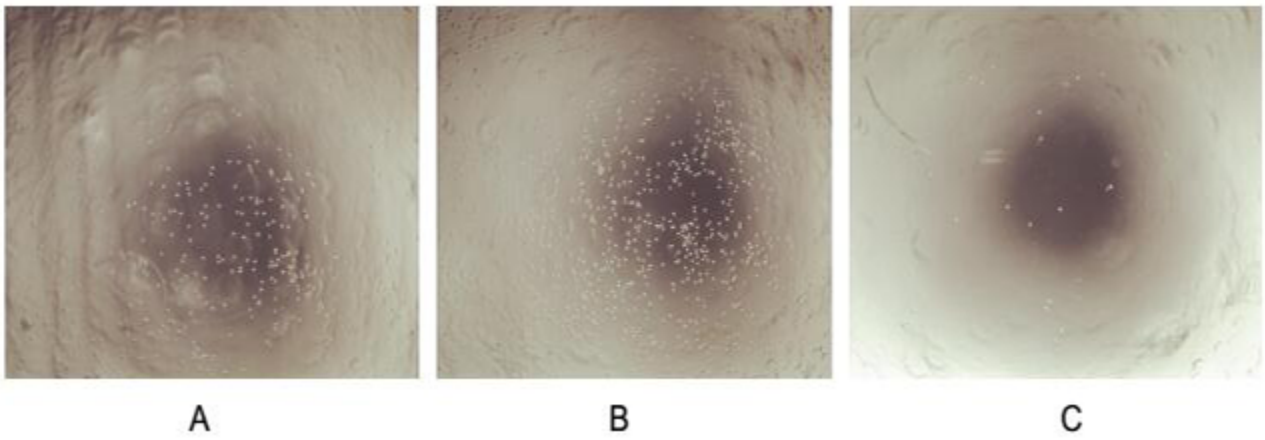
Active Complement Component 5a (C5a) Instruction Manual

SBPA122Hu61

Homo sapiens (Human)

Buffer Formulation	20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01% SKL, 5% Trehalose and Proclin300.
Traits	Freeze-dried powder
Purity	> 95%
Isoelectric Point	8.9
Applications	Cell culture; Activity Assays.

ACTIVITY TEST



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, 106 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 37oC with 5% CO2 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) 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. The chemotactic effect of C5a on THP-1 cells.

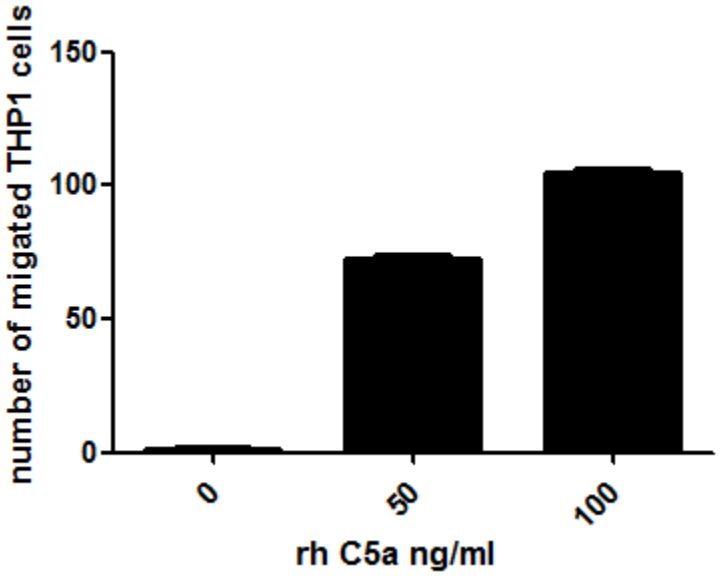


Figure. The chemotactic effect of C5a on THP-1 cells

USAGE

Reconstitute in 20mM Tris, 150mM NaCl (PH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

STORAGE

Avoid repeated freeze/thaw cycles. Store at 2-8°C for one month. Aliquot and store at -80°C for 12 months.

STABILITY

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.

Image

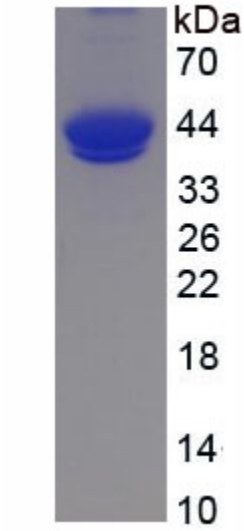


Figure. SDS-PAGE

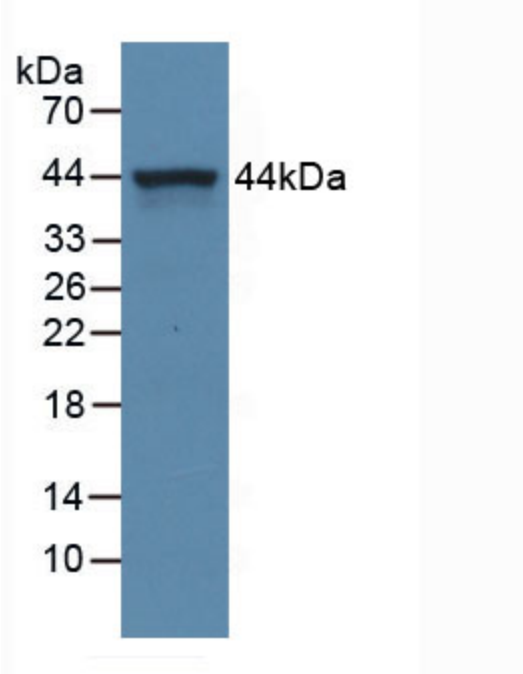


Figure. Western Blot

[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.