Active Interleukin 8 (IL8) Instruction Manual

SBPA052Hu01

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	9.4
Applications	Cell culture; Activity Assays.
Residues	Ser28~Ser99
Tags	N-terminal His-tag

ACTIVITY TEST

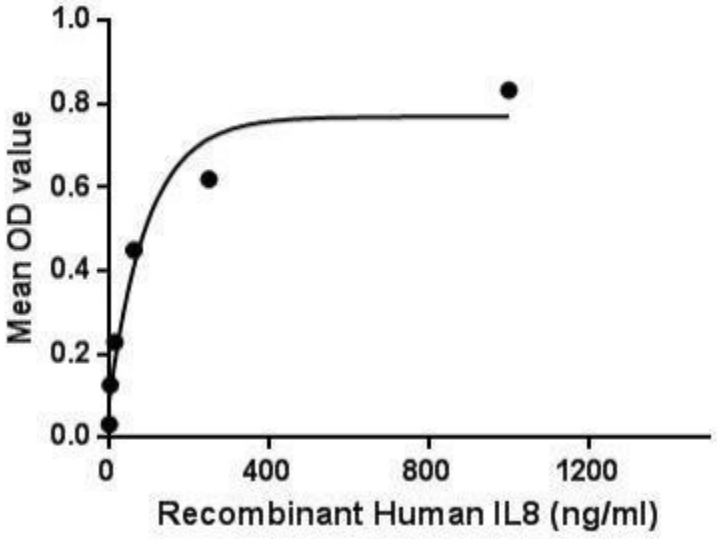

Figure. The binding activity of IL8 with SDC1.

Interleukin 8 (IL8 or chemokine (C-X-C motif) ligand 8, CXCL8) is a chemokine produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cellsendothelial cells. IL-8, also known as neutrophil chemotactic factor, has two primary functions. It induces chemotaxis in target cells, primarily neutrophils but also other granulocytes, causing them to migrate toward the site of infection. IL8 also induces phagocytosis once they have arrived. IL8 is also known to be a potent promoter of angiogenesis. Besides, Syndecan 1 (SDC1) has been identified as an interactor of IL8, thus a binding ELISA assay was conducted to detect the interaction of recombinant human IL8 and recombinant human SDC1. Briefly, IL8 were diluted serially in PBS,

with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to SDC1-coated microtiter wells and incubated for 2h at 37℃. Wells were washed with PBST and incubated for 1h with anti-IL8 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50μL stop solution to the wells and read at 450nm immediately. The binding activity of IL8 and SDC1 was shown in Figure 1, and this effect was in a dose dependent manner.

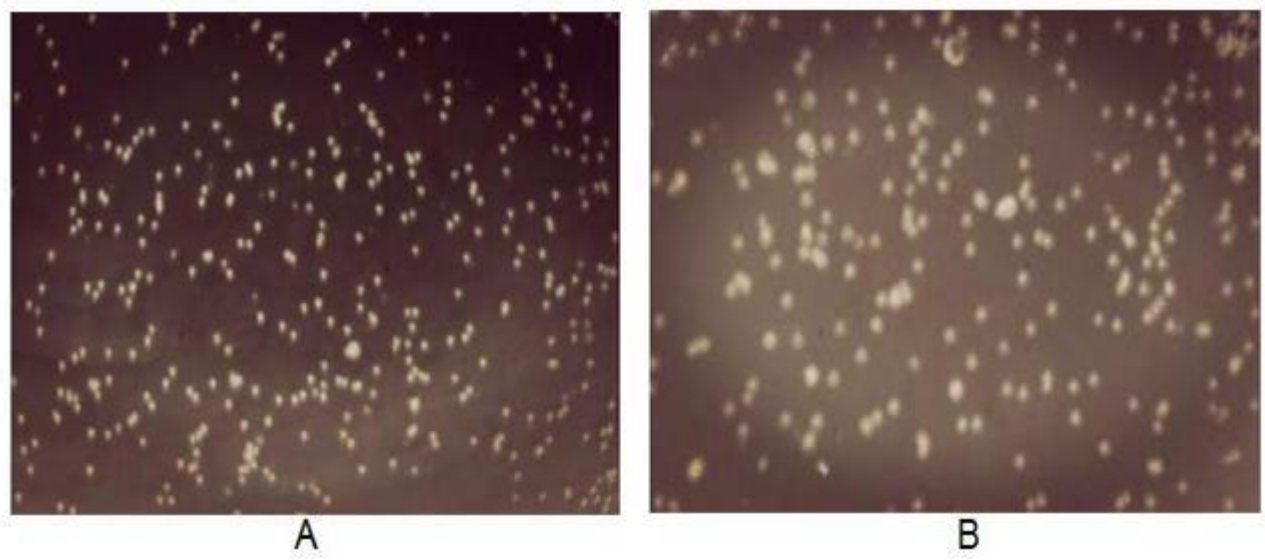

Figure. The chemotactic effect of IL8 on Jurkat cells

IL8 is a kind of neutrophil chemotactic factor. Thus, chemotaxis assay used 24-well microchemotaxis system was undertaken to detect the chemotactic effect of IL8 on the human T-lymphocyte leukemia cell line Jurkat. Briefly, Jurkat cells were seeded into the upper chambers (100μL cell suspension, 106cells/mL in RPMI 1640 with FBS free) and recombinant human IL8 (10ng/mL, 100ng/mL and 1000ng/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℃ with 5% CO2 for 1h, 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 shows IL8 is able to induce migration of Jurkat cells. The migrated Jurkat cells in low chamber at low magnification (×100) were shown in Figure 2. Five fields of each chamber were randomly chosen, and the migrated cells were counted at high magnification (×400). Statistical results were shown in Figure 3. The optimum chemotaxis of IL8 occurs at 10~100ng/mL.

(A) Jurkat cells were seeded into the upper chambers and serum free RPMI 1640 with 10ng/mL IL8 was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation for 1h;

(B) Jurkat cells were seeded into the upper chambers and serum free RPMI 1640 without

IL8 was added in lower chamber, then cells in lower chamber were observed at low magnification (×100) after incubation for 1h.

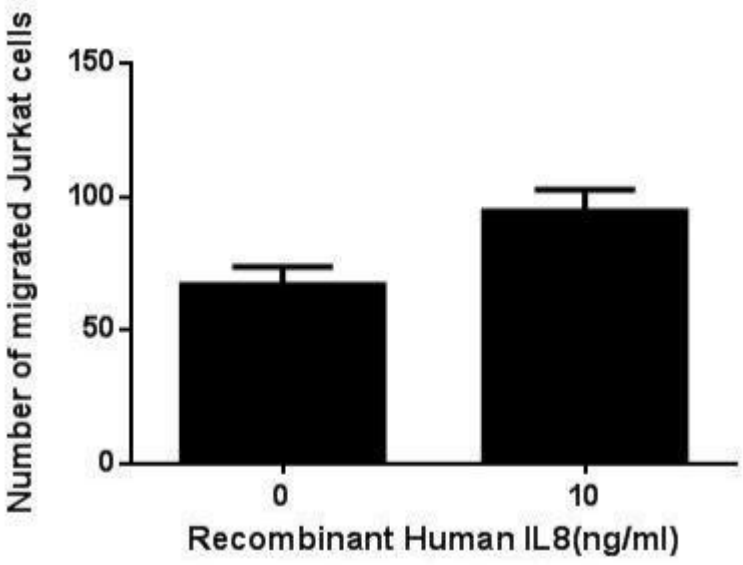

Figure. The chemotactic effect of IL8 on Jurkat cells.

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

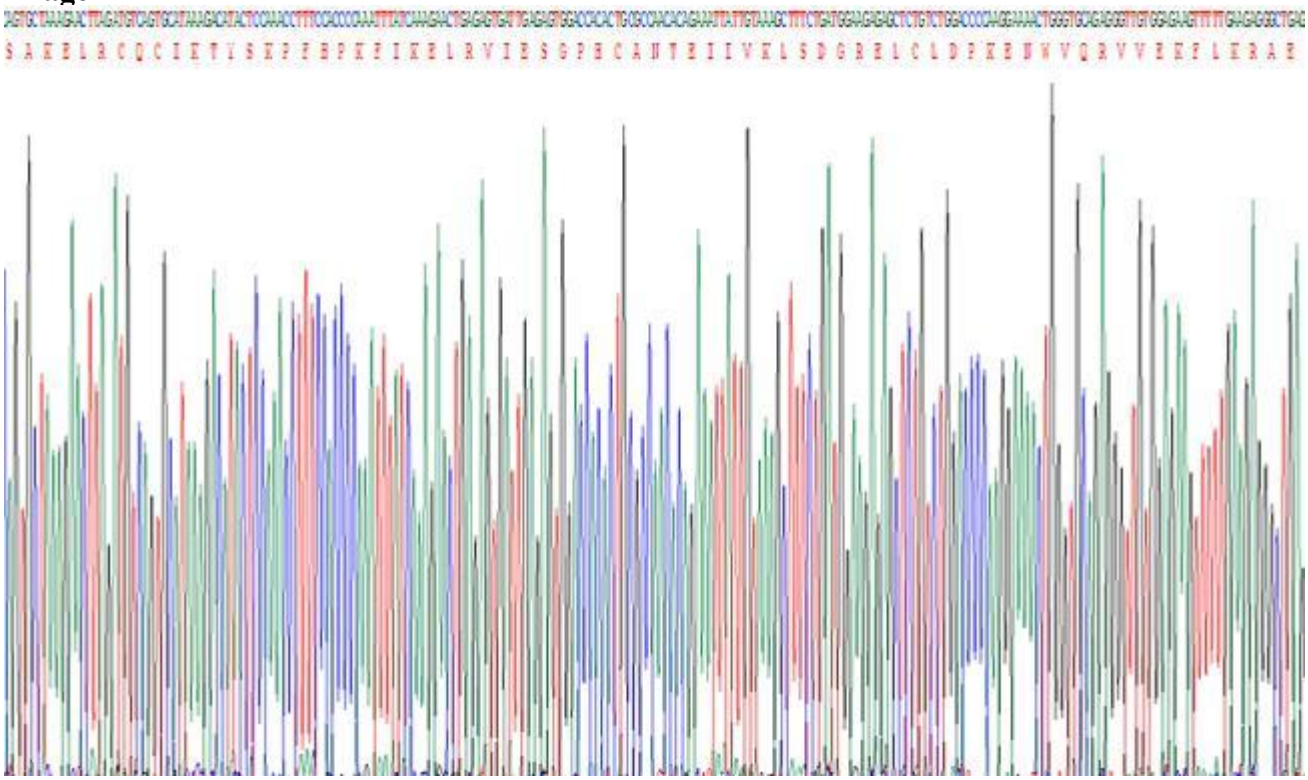

Figure. Gene Sequencing (Extract)

Image

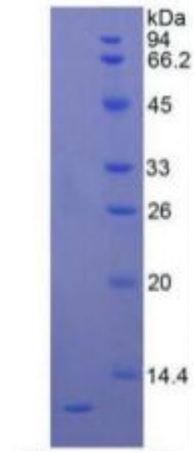

Figure. SDS-PAGE

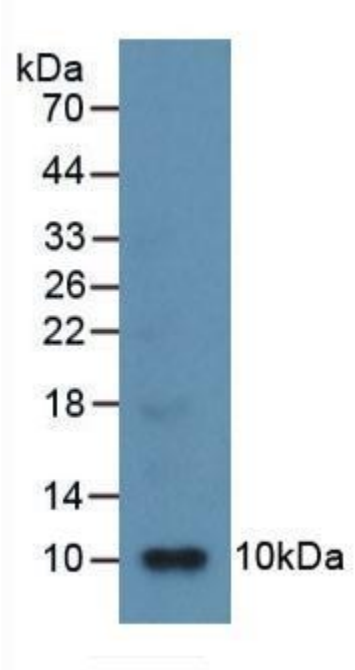

Figure. Western Blot

[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.