APA821Ra01 100μg
Active C Reactive Protein (CRP)
Organism Species: *Rattus norvegicus (Rat)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: His20~Ser230
Tags: N-terminal His-tag
Purity: >95%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 5.7
Predicted Molecular Mass: 27.0kDa
Accurate Molecular Mass: 27kDa as determined by SDS-PAGE reducing conditions.

[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]

H EDMSKQAFVF PGVSATAYVS LEAESKKPLE
AFTVCLYAHA DVSRSFSIFS YATKTSFNEI LLFWTRGQGF SIAVGGPEIL
FSASEIPEVP THICATWESA TGIVELWLDG KPRVRKSLQK GYIVGTNASI
ILGQEQDSYG GGFDANQSLV GDIGDVNMWD FVLSPEQINA VYVGRVFSPN
VLNWRALKYE THGDVFIKPQ LWPLTDCCES

[ACTIVITY]

C-reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via C1q.Besides,CRP have been proved can promote migration of HepG2 cells, 5×104 cells were seeded into 6 well plates. After cell confluent, using a (yellow) pipette tip make a straight scratch, simulating a wound, then washing the wells three times with PBS. Adding 1% serum standard DMEM containing various concentrations of recombinan rat CRP to each well, incubating the plate for 48 hours at 37℃, 5% CO2. Use Image J to measure the area of a scratch, then caculate the cell motility with (0harea -48harea)/0harea ×100%. After affect with 62.5ng/ml CRP for 48h, cell motility is 47%,wiithout affect by CRP, the cell motility is 27%. The results observed by inverted microscope was shown in Figure1.

D-

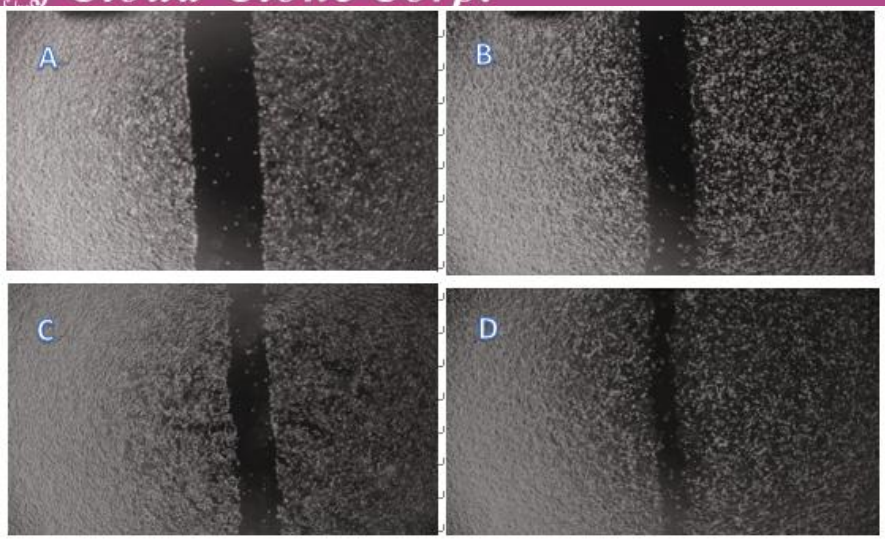

Figure 1. Wound healing assay of HepG2 cells after affect with CRP.

A. HepG2 cells cultured in DMEM without CRP for 0h ;
B. HepG2 cells cultured in DMEM without CRP for 48h;
C. HepG2 cells cultured in DMEM with 62.5ng/ml CRP for 0h;
D. HepG2 cells cultured in DMEM with 62.5ng/ml CRP for 48h

C reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via C1q. Besides, Coagulation Factor II (F2) has been identified as an interactor of CRP, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant rat CRP and recombinant rat F2. Briefly, CRP was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl were then transferred to F2-coated microtiter wells and incubated for 1h at 37℃. Wells were washed with PBST and incubated for 1h with anti-CRP pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37℃, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃.

Finally, add 50 μL stop solution to the wells and read at 450/630 nm immediately. The binding activity of recombinant rat CRP and recombinant rat F2 was shown in Figure 1, the EC50 for this effect is 0.019 ug/mL.

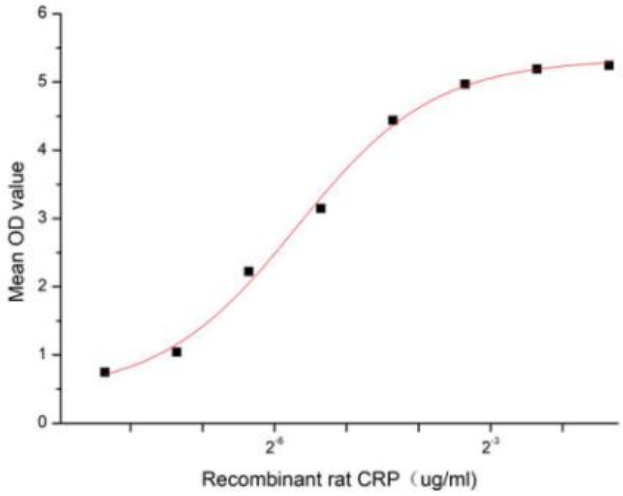

Figure 2. The binding activity of recombinant rat CRP and recombinant rat F2

[IDENTIFICATION]

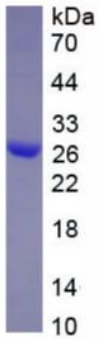

Figure 3. SDS-PAGE

Sample: Active recombinant CRP, Rat

[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.