

APA753Hu02 100µg
Active Toll Like Receptor 4 (TLR4)
Organism Species: *Homo sapiens (Human)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

12th Edition (Revised in Aug, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Cys29~Leu253

Tags: N-terminal His-tag

Purity: >95%

Traits: Freeze-dried powder

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

Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 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: 6.0

Predicted Molecular Mass: 29.0kDa

Accurate Molecular Mass: 29kDa 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]

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CV EVVPNITYQC MELNFYKIPD
NLPFSTKNLD LSFNPLRHLG SYSFFSFPEL QVLDLSRCEI QTIEDGAYQS
LSHLSTLILT GNPIQSLALG AFSGLSSLQK LVAVETNLAS LENFPIGHLK
TLKELNVAHN LIQSFKLPEY FSNLTNLEHL DLSSNKIQSI YCTDLRVLHQ
MPLLNLSDL SLNPMNFIQP GAFKEIRLHK LTLRNNFDSL NVMKTCIQGL
AGL
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[ACTIVITY]

TLR4 is a 100 kDa type I transmembrane glycoprotein that belongs to the mammalian Toll-Like Receptor family of pathogen pattern recognition molecules. TLRs plays a fundamental role in pathogen recognition and activation of innate immunity. [Besides](#), TLR4 is the receptor for LPS and plays a critical role in innate immunity. Stimulation of TLR4 activates proinflammatory pathways and induces cytokine expression in a variety of cell types. Inflammatory pathways are activated in tissues of obese animals and humans and play an important role in obesity-associated insulin resistance. It is reported that RETN has been identified as an interactor of TLR4, thus a binding ELISA assay was conducted to detect the interaction of recombinant human RETN and recombinant human TLR4. Briefly, RETN was diluted serially in PBS with 0.01% BSA (pH 7.4).

Duplicate samples of 100 μ L were then transferred to TLR4-coated microtiter wells and incubated for 1h at 37°C. Wells were washed with PBST and incubated for 1h with anti-RETN pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37°C, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37°C. Finally, add 50 μ L stop solution to the wells and read at 450/630nm immediately. The binding activity of TLR4 and RETN was shown in Figure 1. When recombinant human TLR4 is immobilized at 2 μ g/mL (100 μ L/well), the concentration of rhRETN that produces 50% optimal binding response is found to be approximately 5.13 μ g/mL.

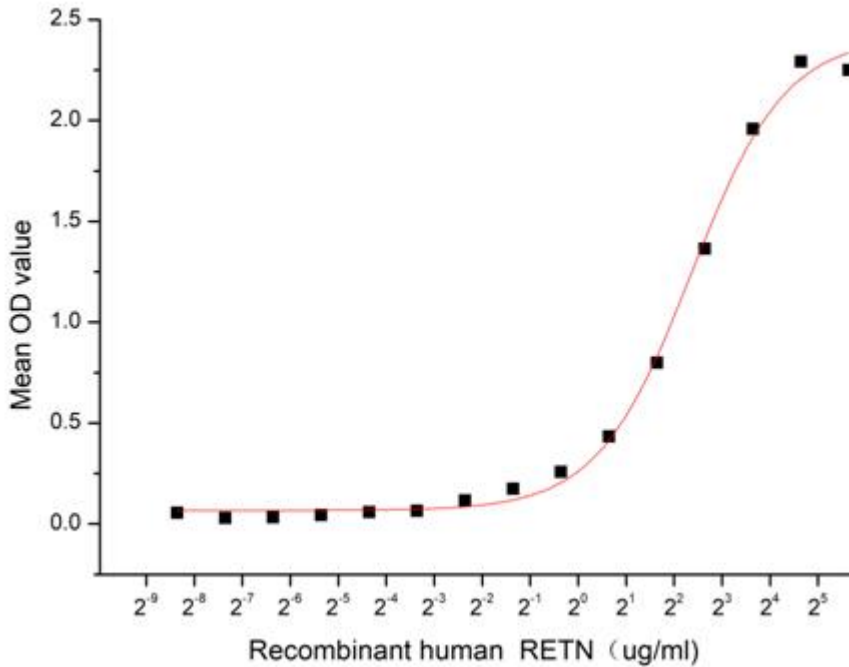


Figure 1. The binding activity of recombinant human RETN with recombinant human TLR4

[IDENTIFICATION]

CGATTCGGGCGGAGCGGCTGTAAATTCCTCTATGAGCAATGAGATTCGCAATTCGCAAAATGCTGGACAGCTCCCTCTCCACAGAGAGCTGGAGCTGGATTTGCTCCAGGCGATTCAGCGAGCTTATGCTCTTCCGTTTCCGAGACTCCAGCTCCGATTTATCCGGGCGGAAATCGAGATTGAGATCCCGGATATGAGAGCTTACCGCC

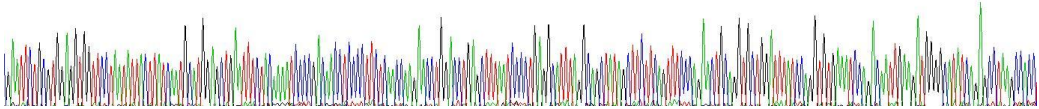


Figure 2. Gene Sequencing (extract)

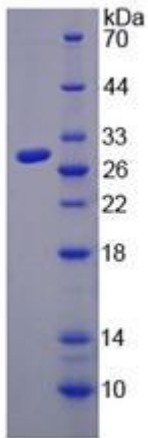


Figure 3. SDS-PAGE

Sample: Active recombinant TLR4, Human

[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.