APA753Hu01 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: Phe326~Ile634

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: 5.8

Predicted Molecular Mass: 42.0kDa

Accurate Molecular Mass: 40kDa 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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                           FSYNF GWQHLELVNC KFGQFPTLKL
KSLKRLTFTS NKGGNAFSEV DLPSLEFLDL SRNGLSFKGC CSQSDFGTTS
LKYLDLSFNG VITMSSNFLG LEQLEHLDFQ HSNLKQMSEF SVFLSLRNLI
YLDISHTHTR VAFNGIFNGL SSLEVLKMAG NSFQENFLPD IFTELRNLTF
LDLSQCQLEQ LSPTAFNSLS SLQVLNMSHN NFFSLDTFPY KCLNSLQVLD
YSLNHIMTSK KQELQHFPSS LAFLNLTQND FACTCEHQSF LQWIKDQRQL
LVEVERMECA TPSDKQGMPV LSLNITCQMN KTII
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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 μl were then transferred to TLR4-coated microtiter wells and incubated for 1h at 37℃.

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 ℃ , 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 450/630nm immediately. The binding activity of TLR4 and RETN was shown in Figure 1. When recombinant human TLR4 is Immobilized at 2 ug/mL (100 uL/well), the concentration of rhRETN that produces 50% optimal binding response is found to be approximately 6.25 ug/mL.
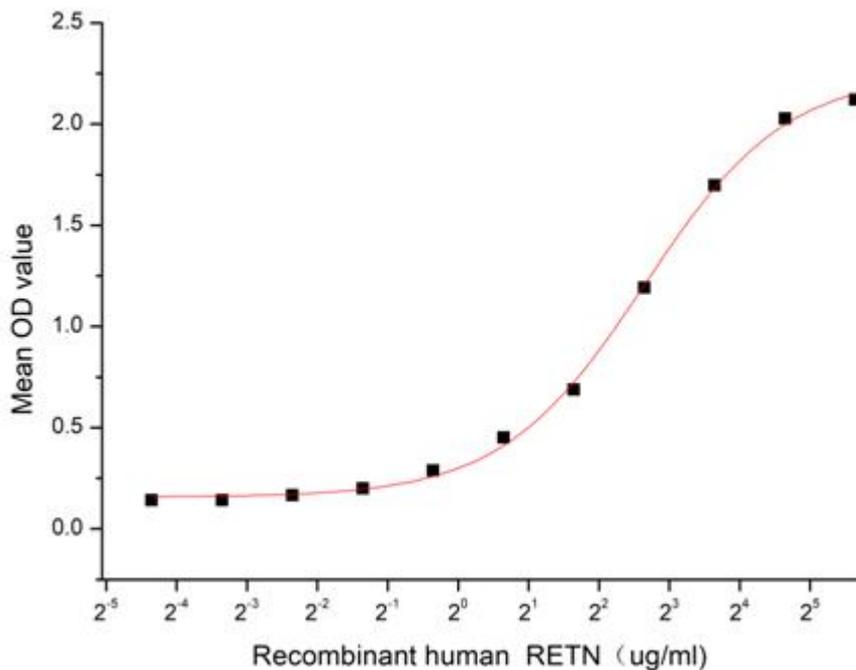

Figure 1. The binding activity of recombinant human RETN with recombinant humanTLR4
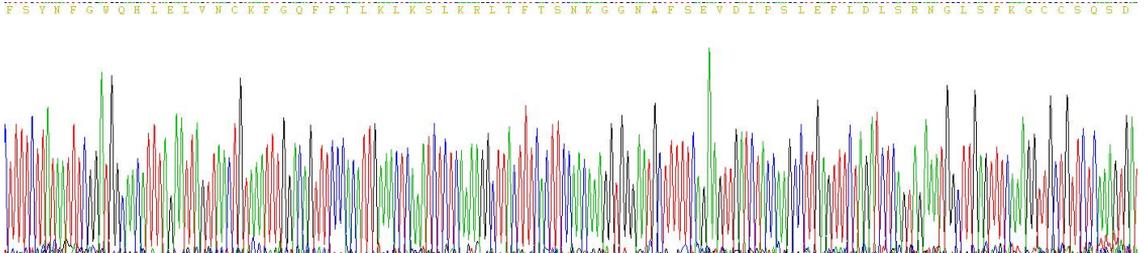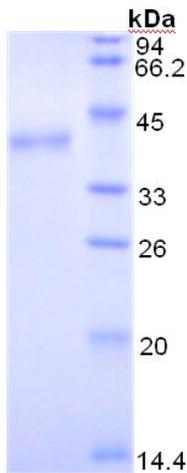
[IDENTIFICATION]

F S Y N F G W Q H L E L V N C K F G Q F P T L K L K S L K R L T F T S N K G G N A F S E V D L P S L E F L D L S R N G L S F K G C C S Q S D

Figure 2. Gene Sequencing (extract)

15% SDS-PAGE

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.