

APA072Hu01 50µg
Active Defensin Beta 2 (DEFb2)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Gly24~Pro64

Tags: N-terminal His and GST 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: 8.0

Predicted Molecular Mass: 34.3kDa

Accurate Molecular Mass: 35kDa 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]

GIGDPVT CLKSGAICHP VFCPRRYKQI
GTCGLPGTKC CKKP

[ACTIVITY]

Defensin Beta 2 (DEFb2) is a 7 kDa secreted protein. It exhibits antimicrobial activity against Gram-negative bacteria and Gram-positive bacteria, with highest activity against Gram-negative bacteria. Antimicrobial activity against *P.aruginosa* seems to be salt-sensitive and is reduced with high salt concentrations greater than 25 mM. The human DEFb2 cDNA encodes a 64 aa protein with a 23 aa signal sequence and a 41 aa mature sequence. Besides, Toll Like Receptor 4 (TLR4) has been identified as an interactor of DEFb2, thus a binding ELISA assay was conducted to detect the interaction of recombinant human DEFb2 and recombinant human TLR4. Briefly, DEFb2 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100µl 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-DEFb2 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 50µL stop solution to the wells and read at 450/630nm immediately. The binding activity of DEFb2 and TLR4 was shown in Figure 1, the EC50 for this effect is 0.11 ug/mL.

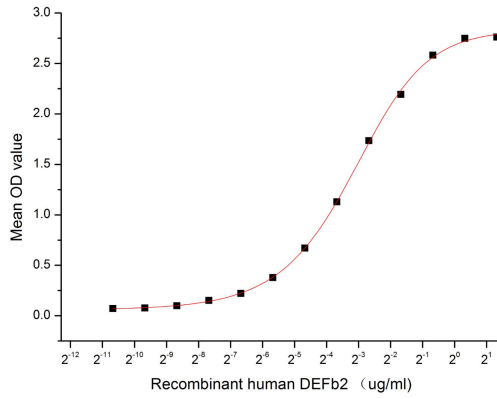


Figure 1. The binding activity of DEFb2 and TLR4

[IDENTIFICATION]

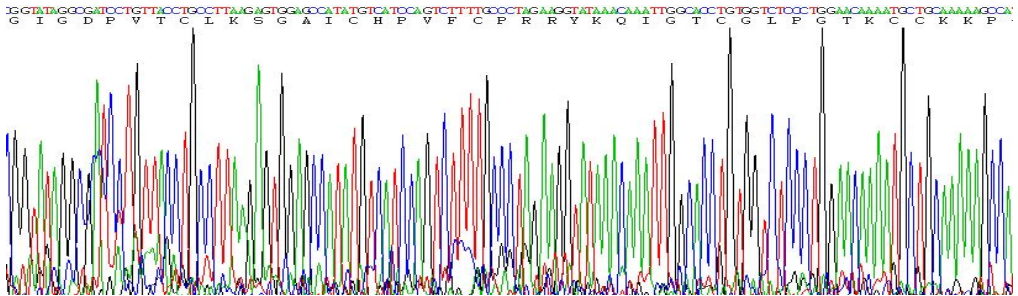


Figure 2. Gene Sequencing (extract)

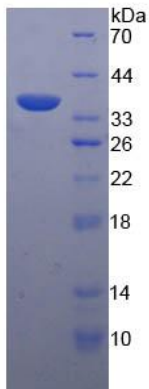


Figure 3. SDS-PAGE

Sample: Active recombinant DEFb2, Human

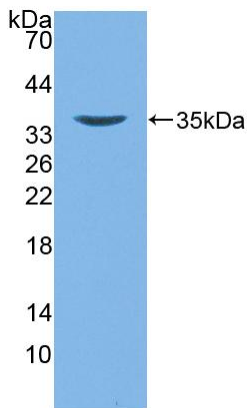


Figure 4. Western Blot

Sample: Recombinant DEFb2, Human;

Antibody: Rabbit Anti-Human DEFb2 Ab (PAA072Hu01)

[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.