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APA072Hu01 10µg 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-tag Purity: >95% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: 10mM PBS, pH7.4, containing 5% trehalose 0.01% sarcosyl

and Proclin300.

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.

[<u>USAGE</u>]

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.

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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) known as skin-antimicrobial peptide 1 (SAP1) is a cysteine-rich cationic low molecular weight antimicrobial peptide. Defensins form a family of microbicidal and cytotoxic peptides made by neutrophils. Members of the defensin family are highly similar in protein sequence. DEFb2 is produced by a number of epithelial cells and exhibits potent antimicrobial activity against Gram-negative bacteria and Candida, but not Gram-positive S. aureus. Besides, potassium voltage-gated channel, shaker-related subfamily, member 3 (KCNA3) 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 KCNA3. Briefly, DEFb2 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to KCNA3-coated microtiter wells and incubated for 2h 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, wells were aspirated and washed 3 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 450nm immediately. The binding activity of DEFb2 and KCNA3 was shown in Figure 1, and this effect was in a dose dependent manner.

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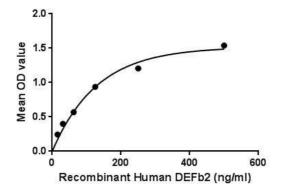
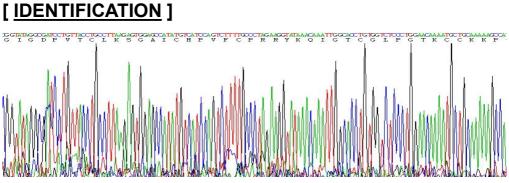


Figure 1. The binding activity of DEFb2 with KCNA3.





-	kDa 70
-	44
-	33 26
-	22
	18
	14
1.1	10
1000	

Figure 3. SDS-PAGE Sample: Active recombinant DEFb2, Human

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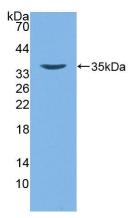


Figure 4. Western Blot Sample: Recombinant DEFb2, Human; Antibody: Rabbit Anti-Human DEFb2 Ab (PAA072Hu01)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.