

APA741Hu01 10µg
Active Dickkopf Related Protein 1 (DKK1)
Organism Species: Homo sapiens (Human)
Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Leu33~His266

Tags: N-terminal His-tag

Purity: >95%

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

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 8.6

Predicted Molecular Mass: 26.9kDa

Accurate Molecular Mass: 33kDa as determined by SDS-PAGE reducing conditions.

Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[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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                                LNSVLNSN AIKNLPPPLG
GAAGHPGSAV SAAPGILYPG GNKYQTIDNY QPYPCAEDDEE CGTDEYCASP
TRGGDAGVQI CLACRKRKR CMRHAMCCPG NYCKNGICVS SDQNHFRGEI
EETITESFGN DHSTLDGYSR RTTLSSKMYH TKQQEGSVCL RSSDCASGLC
CARHFWSKIC KPVLKEGQVC TKHRRKGSYG LEIFQRCYCG EGLSCRIQKD
HHQASNSSRL HTCQRH
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[ACTIVITY]

Dickkopf-related protein 1 (DKK1) is a protein which is a member of the dickkopf family. It is a secreted protein with two cysteine rich regions and is involved in embryonic development through its inhibition of the Wnt signaling pathway. Dickkopf WNT signaling pathway inhibitor 1 (Dkk1) is a protein-coding gene that acts from the anterior visceral endoderm. The dickkopf protein encoded by DKK1 is an antagonistic inhibitor of the WNT signaling pathway that acts by isolating the CTNnb1 co-receptor so that it cannot aid in activating the WNT signaling pathway. DKK1 was also demonstrated to antagonize the Wnt/ β -catenin pathway via a

reduction in β -catenin and an increase in OCT4 expression. Besides, Catenin Beta 1 (CTNNb1) has been identified as an interactor of DKK1 thus a binding ELISA assay was conducted to detect the interaction of recombinant human DKK1 and recombinant human CTNNb1. Briefly, DKK1 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to CTNNb1-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-DKK1 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 50 μ L stop solution to the wells and read at 450nm immediately. The binding activity of DKK1 and CTNNb1 was shown in Figure 1, and this effect was in a dose dependent manner.

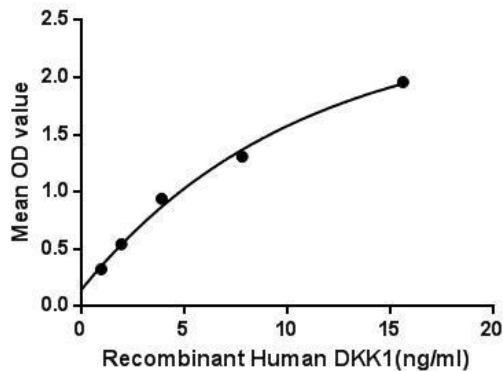


Figure 1. The binding activity of DKK1 with CTNNb1.

[IDENTIFICATION]

INSVLNSNAIKNLPFPLGGGAAGHPGSAVSAAPGILTPGGNKYQTIDNYQYPCAEDEECGTDEYCASPTRGGDAGYQICLACRKRKRRCMRHAMCC

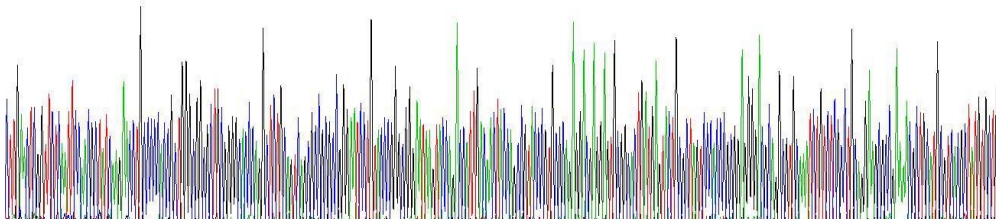


Figure 2. Gene Sequencing (extract)

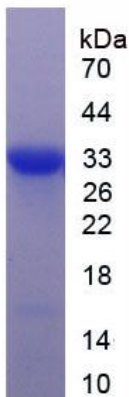


Figure 3. SDS-PAGE

Sample: Active recombinant DKK1, Human

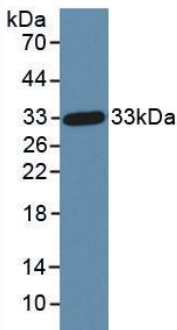


Figure 4. Western Blot

Sample: Recombinant DKK1, Human;

Antibody: Rabbit Anti-Human DKK1 Ab (PAA741Hu01)