

APA058Hu01 10µg
Active Interleukin 12B (IL12B)
Organism Species: *Homo sapiens (Human)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Val30~Ser320

Tags: N-terminal His-tag

Purity: >96%

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

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.01% sarcosyl and 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: 5.5

Predicted Molecular Mass: 36.6kDa

Accurate Molecular Mass: 44kDa 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 20mM Tris, 150mM NaCl (pH8.0) 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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                                         V YVELDWYPD APGEMVVLTC
DTPEEDGITW TLDQSSEVLG SGKTLTIQVK EFGDAGQYTC HKGGEVLSHS
LLLLHKKEDG IWSTDILKDQ KEPKNKTLR CEAKNYSGRF TCWWLTTIST
DLTFSVKSSR GSSDPQGVTC GAATLSAERV RGDNKEYEYS VECQEDSACP
AAEESLPIEV MVDVAVHKLKY ENYTSSFFIR DIIKPDPPKN LQLKPLKNSR
QVEVSWEYPD TWSTPHSYFS LTFCVQVQGK SKREKKDRVF TDKTSATVIC
RKNASISVRA QDRYYSSWS
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[ACTIVITY]

Interleukin 12B (IL12B) encodes a subunit of interleukin 12, a cytokine that acts on T and natural killer cells, and has a broad array of biological activities. Interleukin 12 is a disulfide-linked heterodimer composed of the 40 kD cytokine receptor like subunit encoded by this gene, and a 35 kD subunit encoded by IL12A. This cytokine is expressed by activated macrophages that serve as an essential inducer of Th1 cells development. Interleukin 12B can combine with Interleukin 12 Receptor Beta 1 (IL12Rb1). Thus a binding ELISA

assay was conducted to detect the interaction of recombinant human IL12B and recombinant human IL12Rb1. Briefly, biotin-linked recombinant human IL12B were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100µl then transferred to IL12Rb1-coated microtiter wells and incubated for 1h at 37 °C . Wells were washed with PBST 3 times and incubation with HRP conjugage for 30min, then 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 450nm immediately. The binding activity of IL12B and IL12Rb1 was shown in Figure 1, and this effect was in a dose dependent manner.

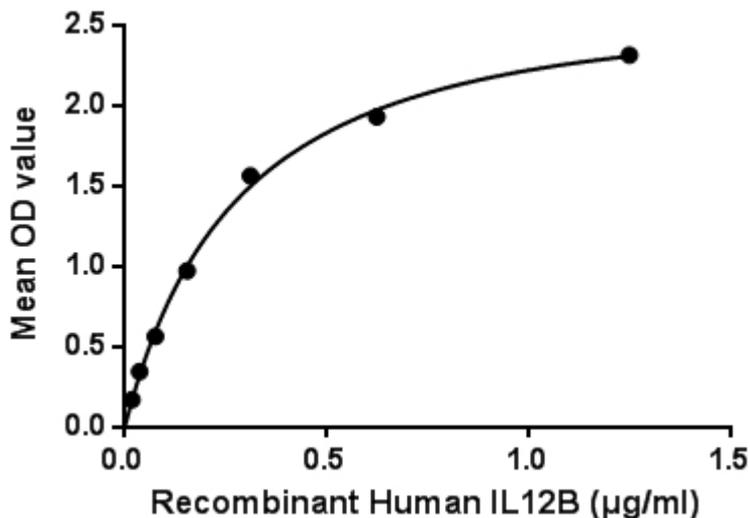


Figure 1. The binding activity of IL12B with IL12Rb1

[IDENTIFICATION]

GGATCCGTTTATGTCGTA GAATTGGAT TGGTATCC GGATGCCCTGGAGAAATGGTGGTCTCACCTGTGACACCCCTG AAGAAGATGGTATCACCTG GACCTTGGACAGAGCA GTGAGGTCTTAGGCCTGGCAAA

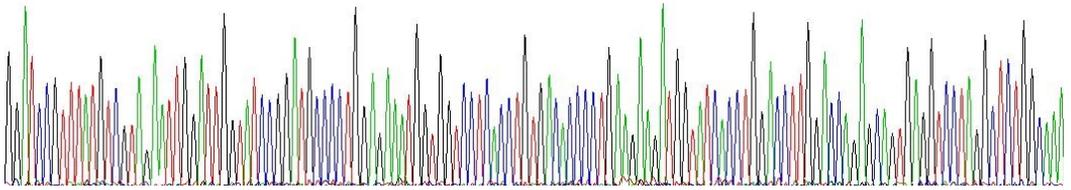


Figure 2. Gene Sequencing (extract)

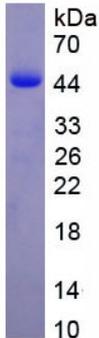


Figure 3. SDS-PAGE

Sample: Active recombinant IL12B, Human

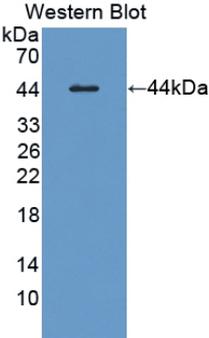


Figure 4. Western Blot

Sample: Recombinant IL12B, Human;

Antibody: Rabbit Anti-Human IL12B Ab (PAA058Hu01)

[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.