APB547Hu01 100µg Active Indoleamine-2,3-Dioxygenase (IDO) 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: Ala2~Gly403

Tags: N-terminal His-tag

Purity: >98%

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.

Applications: Cell culture; Activity Assays; In vivo assays.

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

Predicted isoelectric point: 6.8

Predicted Molecular Mass: 46.7kDa

Accurate Molecular Mass: 43kDa 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.

[<u>USAGE</u>]

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.

[<u>SEQUENCE</u>]

AHAMENSWT ISKEYHIDEE VGFALPNPQE NLPDFYNDWM FIAKHLPDLI ESGQLRERVE KLNMLSIDHL TDHKSQRLAR LVLGCITMAY VWGKGHGDVR KVLPRNIAVP YCQLSKKLEL PPILVYADCV LANWKKKDPN KPLTYENMDV LFSFRDGDCS KGFFLVSLLV EIAAASAIKV IPTVFKAMQM QERDTLLKAL LEIASCLEKA LQVFHQIHDH VNPKAFFSVL RIYLSGWKGN PQLSDGLVYE GFWEDPKEFA GGSAGQSSVF QCFDVLLGIQ QTAGGGHAAQ FLQDMRRYMP PAHRNFLCSL ESNPSVREFV LSKGDAGLRE AYDACVKALV SLRSYHLQIV TKYILIPASQ QPKENKTSED PSKLEAKGTG GTDLMNFLKT VRSTTEKSLL KEG

[ACTIVITY]

IDO (Indoleamine 2,3-dioxygenase 1) is a heme enzyme that catalyzes the first and rate-limiting step in tryptophan catabolism to N-formyl-kynurenine. This enzyme acts on multiple tryptophan substrates including D-tryptophan, L-tryptophan, 5-hydroxy-tryptophan, tryptamine, and serotonin. Thus, bioactivity of recombinant human IDO was measured through its ability to oxidize L-tryptophan

to N-formyl-kynurenine, using Methylene Blue as indicator. Briefly, a mixture of 800µM L-tryptophan, 9000 units/mL catalase, and 40µM Methyene Blue with the addition of 80mM ascorbic acid reaction buffer was incubated with different concentrations of IDO, and the absorbance was read in 321nm every 1.5h. The result indicated that recombinant human IDO oxidize L-tryptophan.

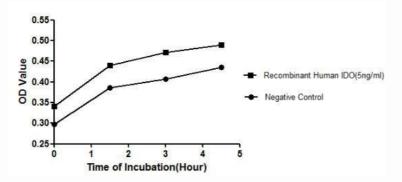


Figure 1. Enzyme activity of recombinant human IDO.

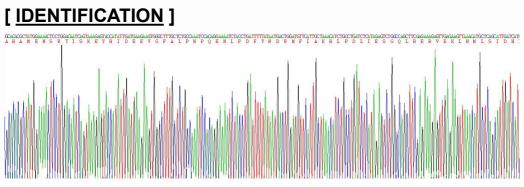


Figure 2. Gene Sequencing (extract)

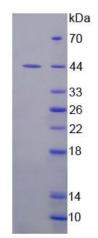


Figure 3. SDS-PAGE

Sample: Active recombinant IDO, Human

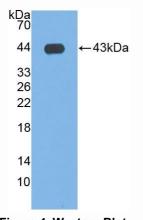


Figure 4. Western Blot

Sample: Recombinant IDO, Human;

Antibody: Rabbit Anti-Human IDO Ab (PAB547Hu01)