APB547Hu01 200μg
Active Indoleamine-2,3-Dioxygenase (IDO)
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: Ala2~Gly403

Tags: N-terminal His-tag

Purity: >98%

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

Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.

Original Concentration: 600μg/mL

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.

[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]

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. The reaction was performed in 50 mM MES, pH 6.5 (Assay Buffer), initiated by

addition 50 μL of various concentrations of IDO (diluted by Assay Buffer) to 50 μL substrate mixture of 800 μM L-tryptophan, 9000 units/mL catalase （RPC418Hu05）, and 40 μM Methyene Blue in assay buffer with equal volume of 80 mM ascorbic acid in 0.405 M Tris, pH 8.0. The final well serves as a negative control with no IDO, replaced with 50μL assay buffer. The absorbance was read in 321 nm in kinetic mode for 5 minutes. The result indicated that recombinant human IDO can oxidize L-tryptophan, the specific activity is 10581 pmol/min/μg.

$$\text{Specific Activity (pmol/min/ug)} = \frac{\text{Adjusted } V_{max}^{*} \text{ (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol}}{\text{ext. coeff}^{**} \text{ (M}^{-1}\text{cm}^{-1}\text{) x path corr.}^{***} \text{ (cm) x amount of enzyme (ug)}}$$

*Adjusted for Substrate Blank
**Using the extinction coefficient 3750 $M^{-1}cm^{-1}$
***Using the path correction 1 cm

[IDENTIFICATION]

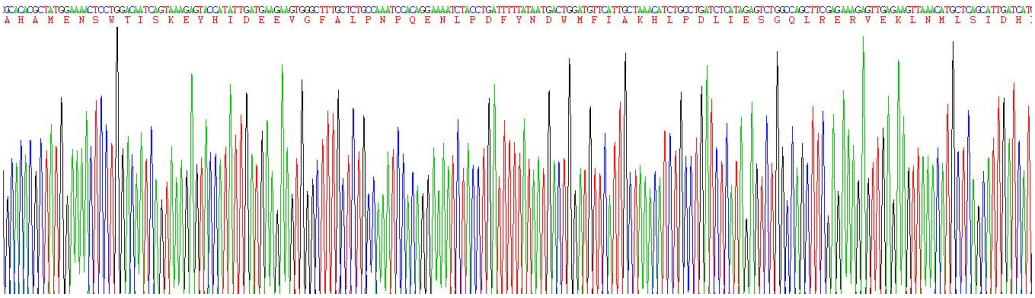

Figure 1. Gene Sequencing (extract)

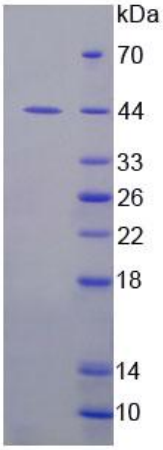

Figure 2. SDS-PAGE

Sample: Active recombinant IDO, Human

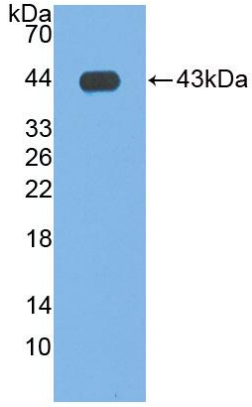

Figure 3. Western Blot

Sample: Recombinant IDO, Human;

Antibody: Rabbit Anti-Human IDO Ab (PAB547Hu01)

[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.