APA054Hu02 2mg Active Insulin Like Growth Factor Binding Protein 3 (IGFBP3) Organism Species: *Homo sapiens* (Human) *Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Gly135~Thr277

Tags: Two N-terminal Tags, His-tag and SUMO-tag

Purity: >95%

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

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

Original Concentration: 5000µg/mL

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 9.3

Predicted Molecular Mass: 30.0kDa

Accurate Molecular Mass: 34kDa 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 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.

[<u>SEQUENCE</u>]

GNASES EEDRSAGSVE SPSVSSTHRV SDPKFHPLHS KIIIIKKGHA KDSQRYKVDY ESQSTDTQNF SSESKRETEY GPCRREMEDT LNHLKFLNVL SPRGVHIPNC DKKGFYKKKQ CRPSKGRKRG FCWCVDKYGQ PLPGYTT

[ACTIVITY]

Insulin-like growth factor-binding protein 3, also known as IGFBP3 is one of six IGF binding proteins (IGFBP1 to IGFBP6) that have highly conserved structures and bind the insulin-like growth factors IGF-1 and IGF-2 with high affinity. Within tissues, IGFBP3 can bind IGF1 and IGF2 released by many cell types, and block their access to the IGF-1 receptor (IGF1R), which is activated by both IGFs. IGFBP3 also interacts with cell-surface proteins, affecting cell signaling from outside the cell or after internalization, and also enters the cell nucleus where it binds to nuclear hormone receptors and other ligands. Besides, Fibronectin (FN) has been identified as an interactor of IGFBP3, thus a binding ELISA assay was conducted to detect the interaction of recombinant human IGFBP3 and recombinant human FN.

Briefly, IGFBP3 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100ul were then transferred to FN-coated microtiter wells and incubated for 1h at 37 $^{\circ}$ C. Wells were washed with PBST and incubated for 1h with anti-IGFBP3 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 5 times. With the addition of substrate solution , wells were incubated 15-25 minutes at 37 $^{\circ}$ C. Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of IGFBP3 and FN was shown in Figure 1, the EC50 was 78 ng/ml.

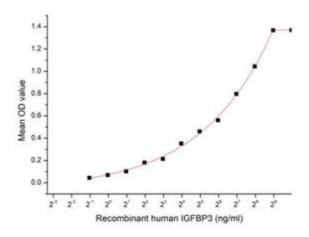


Figure 1. The binding activity of IGFBP3 with FN

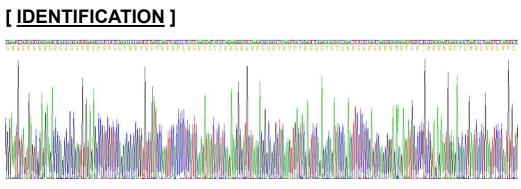


Figure 2. Gene Sequencing (extract)

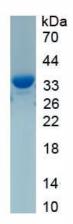


Figure 3. SDS-PAGE

Sample: Active recombinant IGFBP3, Human

[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.