APH776Hu03 100μg
Active Inter Alpha-Globulin Inhibitor H4 (ITIH4)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Ala683~Val928
Tags: N-terminal His-tag
Purity: >98%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% 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: 6.9
Predicted Molecular Mass: 28.9kDa
Accurate Molecular Mass: 33kDa as determined by SDS-PAGE reducing conditions.
Note: 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]

AYHPFRRL AILPASAPPA
TSNPDPAVSR VMNMKIEETT MTTQTPAPIQ APSAILPLPG QSVERLCVDP
RHRQGPVNLL SDPEQGVEVT GQYEREKAGF SWIEVTFKNP LVWVHASPEH
VVVTRNRRSS AYKWKETLFS VMPGLKMTMD KTGLLLLSDP DKVTIGLLFW
DGRGEGLRLL LRDTDRFSSH VGGTLGQFYQ EVLWGSPAAS DDGRRTLRVQ
GNDHSATRER RLDYQEGPPG VEISCWSV

[ACTIVITY]

Inter Alpha-Globulin Inhibitor H4 (ITIH4) is secreted into the blood, where it is cleaved by plasma kallikrein into two smaller forms. ITIH4 has been detected only in liver, and it seems to be upregulated during surgical trauma. It may also play a role in liver development or regeneration. Besides, Growth Factor Receptor Bound Protein 2 (Grb2) has been identified as an interactor of ITIH4, thus a binding ELISA assay was conducted to detect the interaction of recombinant human ITIH4 and recombinant human Grb2. Briefly, ITIH4 were diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to

Grb2-coated microtiter wells and incubated for 2h at 37℃. Wells were washed with PBST and incubated for 1h with anti-ITIH4 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℃. Finally, add 50μL stop solution to the wells and read at 450nm immediately. The binding activity of ITIH4 and Grb2 was shown in Figure 1, and this effect was in a dose dependent manner.

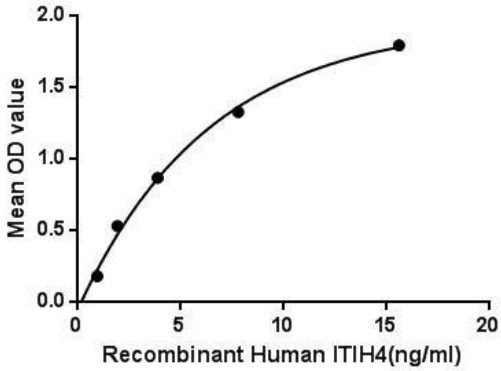

Figure 1. The binding activity of ITIH4 with Grb2.

[IDENTIFICATION]

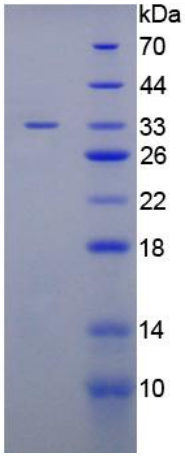

Figure 2. SDS-PAGE

Sample: Active recombinant ITIH4, Human

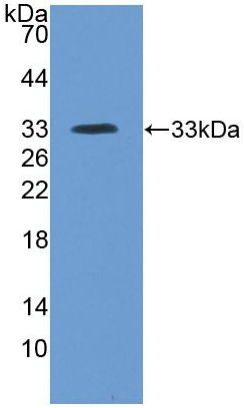

Figure 3. Western Blot

Sample: Recombinant ITIH4, Human;

Antibody: Rabbit Anti-Human ITIH4 Ab (PAH776Hu03)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.