

APB986Hu61 5mg
Active Hemopexin (HPX)

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: Eukaryotic expression.

Host: 293F cell

Residues: Met1~His462

Tags: C-terminal His-tag

Purity: >95%

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

Buffer Formulation: Supplied as a 0.22µm filtered solution in PBS, pH7.4, containing 5% Trehalose .

Original Concentration: 800µg/mL

Applications: Activity Assays.

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

Predicted isoelectric point: 7.0

Predicted Molecular Mass: 52.5kDa

Accurate Molecular Mass: 77kDa 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]

MARVLGAPVALGLWSLCWSLAIATPLPPTSAHGNVAEGETKPPDPVTERCSDGWSFDAT
TLDDNGTMLFFKGEFVWVKSHKWDRELISERWKNFSPVDAAFRQGHNSVFLIKGDKVW
VYPPEKKEKGYPKLLQDEFPGIPSPLDAAVECHRGECAEGVLLFFQGDREWFWDLATGT
MKERSWPAVGNCSALRWLGRYYCFQGNQFLRFDVPVRGEVPPRYPRDVRDYFMPCPG
RGHGHRNGTGHGNSTHHGPEYMRCSPHLVLSALTSNDHGATYAFSGTHYWRLDTSRD
GWHSWPIAHQWPQGPSAVDAAFSWEEKLYLVQGTQVYVFLTGGYTLVSGYPKRLEKE
VGTPHGIILDSVDAAFICPGSSRLHIMAGRRLWWLDLKSGAQATWTELPWPHEKVDGA
LCMEKSLGPNSCSANGPGLYLIHGPNLYCYS DVEKLNAAKALPQPQNVTSLLGCTH

[ACTIVITY]

Hemopexin (HPX), a 60-kDa plasma glycoprotein encoded by the HPX gene, is predominantly synthesized in the liver and belongs to the hemopexin family. As an acute-phase protein, it exhibits the highest heme-binding affinity in plasma, functioning as a critical heme detoxifier. It sequesters free heme released during hemolysis, preventing oxidative stress and tissue damage, and transports heme to the liver for degradation and iron recycling. HPX also modulates inflammation and maintains iron homeostasis, with its expression upregulated during infection and

injury. HPX does not directly bind CD163; its heme complexes are taken up via CD91, while CD163 primarily recognizes haptoglobin-hemoglobin complexes. Briefly, CD163 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ L were then transferred to HPX-coated microtiter wells and incubated for 1h at 37 $^{\circ}$ C. Wells were washed with PBST and incubated for 1h with anti-CD163 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37 $^{\circ}$ C , 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 50 μ L stop solution to the wells and read at 450/630nm immediately. Measured by its binding ability in a functional ELISA. When recombinant human HPX is Immobilized at 2 μ g/mL(100 μ L well), the concentration of CD163 that produces 50% optimal binding response is found to be approximately 1.697 μ g/mL.

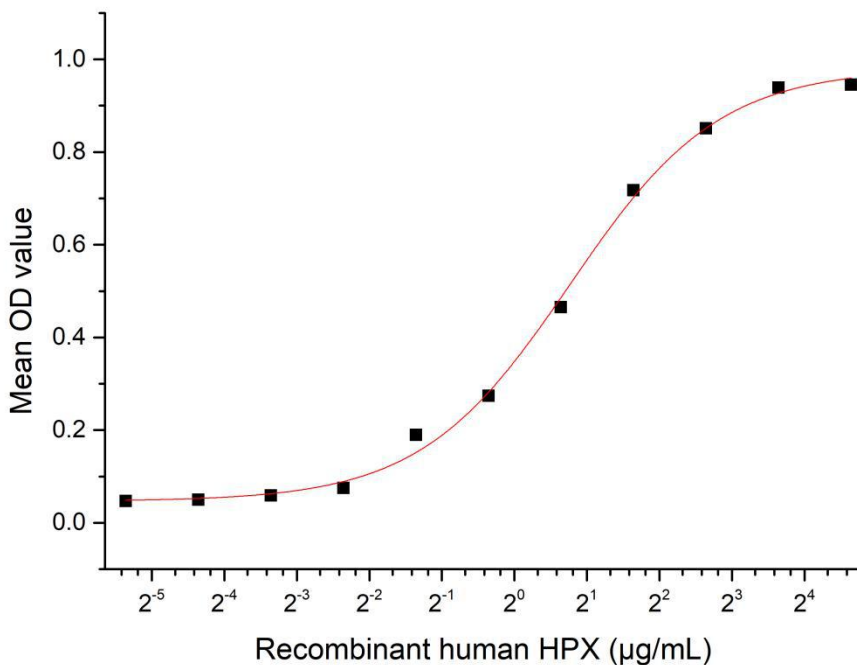


Figure 1. The binding activity of recombinant human HPX and mouse CD163

