

APA817Ra61 100µg
Active Haptoglobin (Hpt)
Organism Species: *Rattus norvegicus (Rat)*
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: Val19~Asn347

Tags: N-terminal His-tag

Purity: >80%

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

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

Original Concentration: 200µg/mL

Applications: Activity Assays.

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

Predicted isoelectric point: 6.3

Predicted Molecular Mass: 38.2kDa

Accurate Molecular Mass: 50&40&16kDa 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]

VELGNDATDIEDDSCPKPPEIANGYVEHLVRYRCRQFYKLQTEGDGIYTLNSEKQWVNP
AAGDKLPKCEAVCGKPKHPVDQVQRIIGGSM DAKGSFPWQAKMISRHGLTTGATLISD
QWLLTTAQNLFNHSENATAKDIAPTLTLYVGNLVEIEKVVLHPERSVVDIGLIKLKQKY
LVTEKVMPICLPSKDYYVAPGRMGYVSGWGRNVNFRFTERLKYVMLPVADQEKCELHYE
KSTVPEKKGAVSPVGVQPILNKHTFCAGLTKYEEDTCYGDAGSAFAVHDT EEDTWYAAGI
LSFDKSCAVAEYGVYVRATDLKDWVQETMAKN

[ACTIVITY]

Haptoglobin (Hpt) is an acute-phase plasma glycoprotein primarily produced by hepatocytes. It serves crucial roles in iron homeostasis and antioxidant defense by binding free hemoglobin (Hb) released during hemolysis, forming an irreversible Hpt-Hb complex. This complex is rapidly cleared via macrophage CD163 receptors, preventing Hb-induced oxidative tissue damage and iron loss. As a sensitive acute-phase reactant, Hpt levels elevate markedly during inflammation, infection, or tissue injury, making it a valuable clinical marker. Notably, Hpt can also bind myoglobin (MYO) - though with lower affinity than Hb - potentially alleviating MYO-mediated oxidative stress in rhabdomyolysis or muscle injury. Thus a

functional binding ELISA assay was conducted to detect the interaction of recombinant rat Hpt and recombinant bovine MYO . Briefly, biotin-linked Hpt were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ l were then transferred to MYO-coated microtiter wells and incubated for 1h at 37 $^{\circ}$ C . Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 30min, then 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 450nm immediately. The binding activity of recombinant rat Hpt and recombinant bovine MYO was shown in Figure 1, the EC50 for this effect is 0.57 μ g/mL.

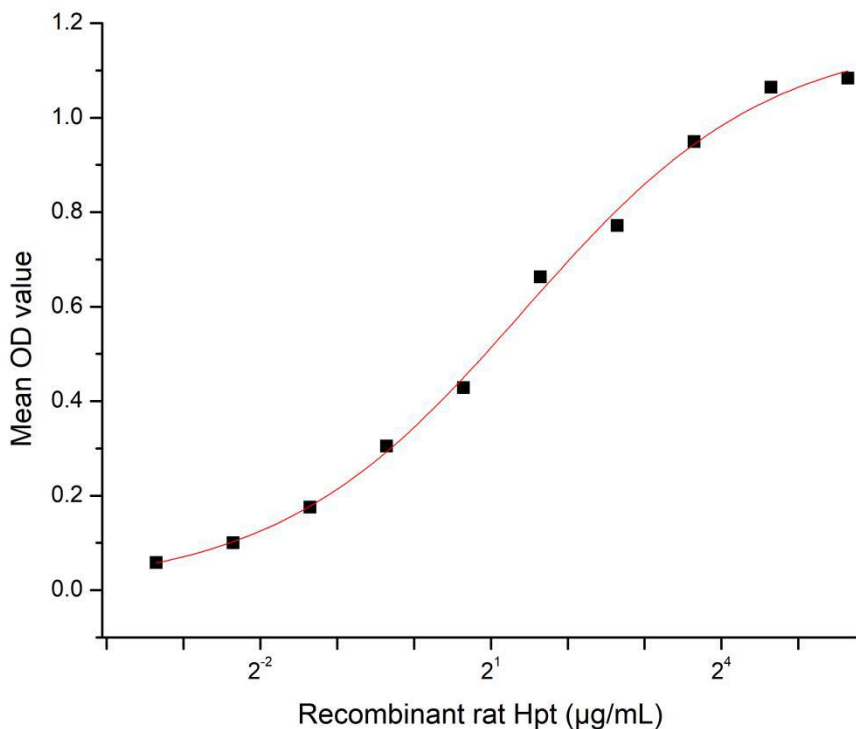


Figure 1. The binding activity of recombinant rat Hpt and recombinant bovine MYO

[IDENTIFICATION]

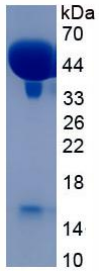


Figure 2. SDS-PAGE

Sample: Active recombinant Hpt, Rat

[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.