

APA284Ra01 100µg

Active Heparin Cofactor II (HCII)

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: Prokaryotic expression.

Host: *E. coli*

Residues: Glu25~Asp235

Tags: N-terminal His-tag

Purity: >95%

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

Buffer Formulation: PBS, pH7.4, containing 0.01% Sarcosyl, 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: 5.5

Predicted Molecular Mass: 28.0kDa

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.

[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]

```

                                EQLTKE NLTVSLPPN FHKENTVTND WIPEGEEDDD
YLDLEKLLSE DDYIYVDA VSPTDSESSA GNILQLFQ GK SRIQLNILN AKFAFNLYRV
LKDQATSSDN IFIAPVGIST AMGMISLGLR GETHEEVHSV LHKDFVNAS SKYEVTTIHN
LFRKLTHRLF RRNFGYTLQS VNDLYIQKQF PIREFKAAM REFYFAEAQE ADFSD
```

[ACTIVITY]

Heparin Cofactor II (HCII) is a plasma serine protease inhibitor synthesized mainly in the liver. Circulating freely in blood plasma, it serves as a key regulator of the coagulation cascade. Unlike antithrombin, HCII selectively suppresses thrombin rather than multiple clotting proteases. Its inhibitory activity remains weak under physiological conditions; binding to glycosaminoglycans such as heparin sodium induces conformational rearrangement of HCII, exposing its reactive site loop to rapidly trap thrombin. HCII limits excessive fibrin generation and prevents pathological thrombosis, maintaining the balance between hemostasis and anticoagulation in the vasculature. HCII binds Hep-Na via positively charged residues, triggering structural shift to accelerate thrombin inhibition. Thus a functional ELISA assay was conducted to detect the interaction of recombinant rat

HCII and Hep-Na. Briefly, HCII was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ L were then transferred to Hep-Na-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-HCII pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37 °C, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 °C. Finally, add 50 μ L stop solution to the wells and read at 450/630nm immediately. The binding activity of recombinant rat HCII and Hep-Na was shown in Figure 1, the EC₅₀ for this effect is 2.56 μ g/mL.

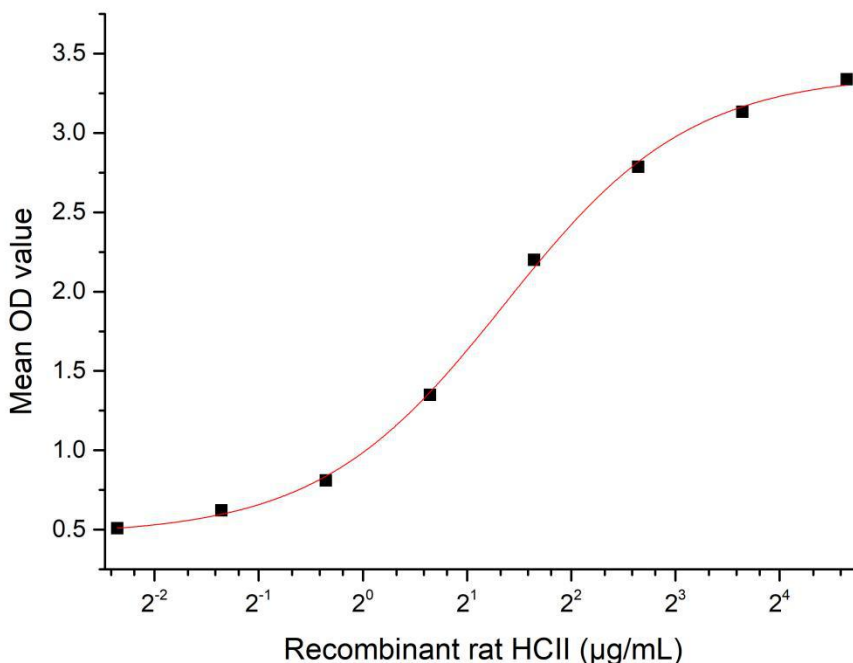


Figure 1. The binding activity of recombinant rat HCII and Hep-Na

[IDENTIFICATION]

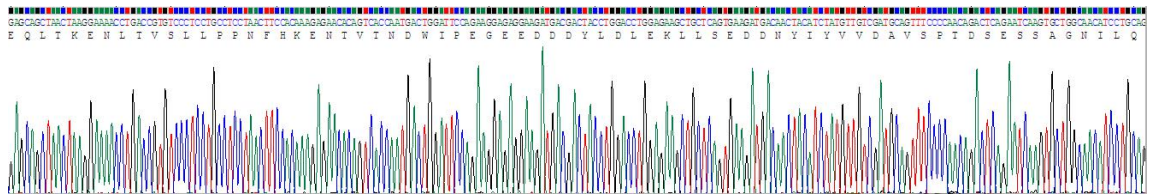


Figure 2. Gene Sequencing (extract)

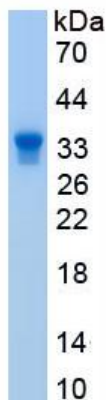


Figure 3. SDS-PAGE

Sample: Active recombinant HCII, 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.