

APC411Hu02 100µg

Active Cubilin (CUBN)

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: Cys3511~Ser3623

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% SKL, 5%Trehalose .

Original Concentration: 200µg/mL

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 5.9

Predicted Molecular Mass: 13.5kDa

Accurate Molecular Mass: 14kDa as determined by SDS-PAGE reducing conditions.

[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]

CGGTLYGDRG SFTSPGYPGT YPNNTYCEWV LVAPAGRLVT
INFYFISIDD PGDCVQNYLT LYDGPNAASP SSGPYCGGDT SIAPFVASSN
QVFIKFHADY ARRPSAFRLT WDS

[ACTIVITY]

Cubilin (CUBN) is a 460 kDa intrinsic factor-vitamin B12 receptor protein and it is expressed in kidney and small intestine. CUBN is a component of a multiligand endocytic receptor complex, which plays a role in lipoprotein, vitamin and iron metabolism by facilitating their uptake. Besides CUBN serves as important transporter in several absorptive epithelia, including intestine, renal proximal tubules and embryonic yolk sac and it plays an important role in the development of the peri-implantation embryo through internalization of APOA1 and cholesterol. Thus a functional binding ELISA assay was conducted to detect the interaction of recombinant human CUBN and recombinant mouse APOA1. Briefly, CUBN was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ l were then transferred to APOA1-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-CUBN 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 450/630 nm immediately. The binding activity of recombinant human CUBN and recombinant mouse APOA1 was

shown in Figure 1, the EC₅₀ for this effect is 0.03 ug/mL.

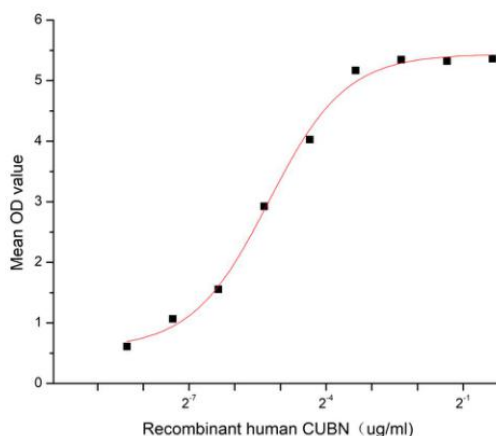


Figure 1. The binding activity of recombinant human CUBN and recombinant mouse APOA1

Cubilin (CUBN) is a large multi-ligand transmembrane glycoprotein primarily expressed in the renal proximal tubules and the apical brush border of intestinal epithelial cells. It functions as a key receptor in nutrient reabsorption and vitamin uptake. By binding to various ligands such as intrinsic factor-vitamin B12 complexes, albumin, and certain lipoproteins, CUBN facilitates their endocytosis and transport, ensuring efficient nutrient retrieval. Mutations in the CUBN gene can lead to malabsorption syndromes, particularly vitamin B12 deficiency, and renal Fanconi syndrome, highlighting its critical role in maintaining systemic nutrient homeostasis and renal function. To detect the activity of recombinant CUBN, a functional ELISA assay was performed to evaluate the interaction between recombinant human CUBN and BSA-vitamin B12. Briefly, CUBN was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl were then transferred to BSA-vitamin B12-coated microtiter wells and incubated for 1h at 37°C. Wells were washed with PBST and incubated for 1h with anti-CUBN 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 µL stop solution to the wells and read at 450/630nm immediately. The

binding activity of recombinant human CUBN and recombinant BSA-vitamin B12 was shown in Figure 1, the EC₅₀ for this effect is 0.176µg/mL.

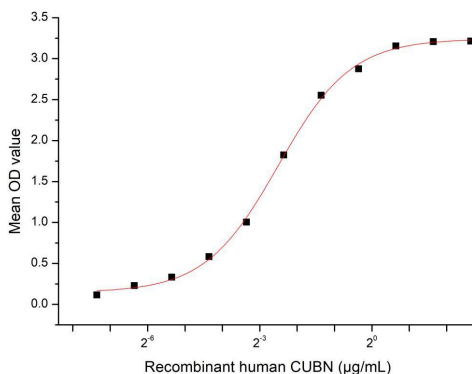


Figure 2. The binding activity of recombinant human CUBN and recombinant BSA-vitamin B12

[IDENTIFICATION]

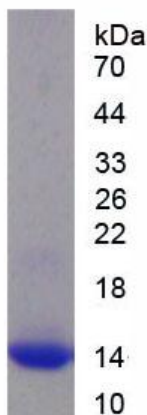


Figure 3. SDS-PAGE

Sample: Active recombinant CUBN, 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.