

**APB007Hu01 100µg**  
**Active Cluster Of Differentiation 320 (CD320)**  
**Organism Species: *Homo sapiens* (Human)**  
***Instruction manual***

FOR RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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13th Edition (Revised in Aug, 2023)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Ser36~Tyr229

**Tags:** N-terminal His-tag

**Purity:** >90%

**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:** 4.1

**Predicted Molecular Mass:** 23.7kDa

**Accurate Molecular Mass:** 30kDa 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.

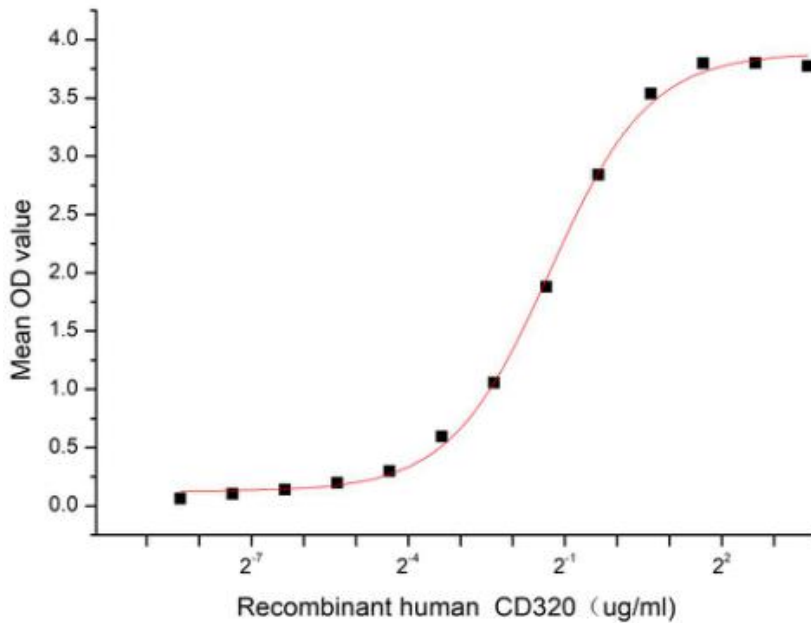
## **[ SEQUENCE ]**

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SPLSTPTSAQAAGPSSGSCPPTKFKCRTSGLCVPLTWRCDRDLDCSDGSDEEEECRIEPC TQKGQC P P P P PGLPCPCTGVSDCSGGTDKCLR  
NCSRLACLAGELRCTLSDDCIPLTWRC DGHPCDPSDELGCGTNEILPEGDATTMGPPVTLESVTLR NATTMGPPVTLESVPSVGNAT  
SSSAGDQSGSPTAY
```

## **[ ACTIVITY ]**

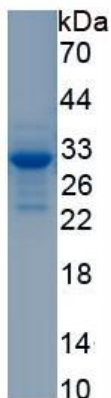
CD320, also known as the Transcobalamin II receptor (TCb1R) and 8D6A, is an approximately 60 kDa transmembrane member of the LDL receptor family. CD320 is expressed in virtually all tissues and is tightly regulated according to the proliferative/differentiation status of the cell and the cellular levels of B12. Cellular uptake of vitamin B12 (cobalamin) requires capture of transcobalamin (TC) from the plasma by CD320. Cubilin (CUBN) is the intrinsic factor-vitamin B12 receptor, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant human CD320 and recombinant human CUBN. Briefly, biotin-linked CD320 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100  $\mu$ l were then transferred to CUBN-coated microtiter wells and incubated for 1h at 37 °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 °C . Finally, add 50 µl stop solution to the wells and read at 450 nm immediately. The binding activity of recombinant human CD320 and recombinant human CUBN was shown in Figure 1, the EC50 for this effect is 0.4 ug/mL.



**Figure 1. The binding activity of recombinant human CD320 and recombinant human CUBN**

**[ IDENTIFICATION ]**



**Figure 2. SDS-PAGE****Sample: Active recombinant CD320, 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.