

**APB249Hu61 100µg**

**Active Tumor Necrosis Factor Receptor Superfamily, Member 5 (CD40)**

**Organism Species: *Homo sapiens (Human)***

***Instruction manual***

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1st Edition (Apr, 2016)

## **[ PROPERTIES ]**

**Source:** Eukaryotic expression.

**Host:** 293F cell

**Residues:** Cys26~Gly187

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

**Purity:** >95%

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

**Buffer Formulation:** PBS, pH7.4, containing 5% trehalose.

**Applications:** Cell culture; Activity Assays.

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

**Predicted isoelectric point:** 4.7

**Predicted Molecular Mass:** 19.5kDa

**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 (pH7.6) 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.

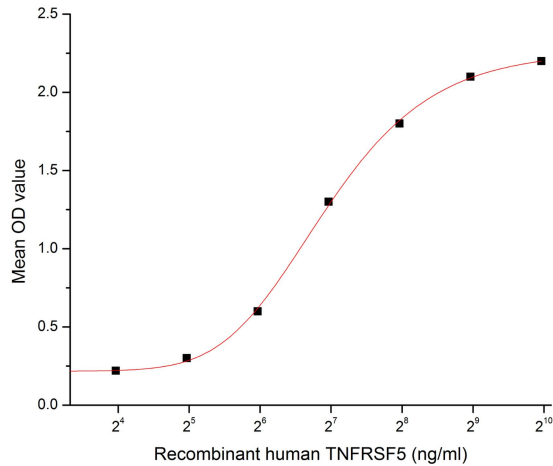
## **[ SEQUENCE ]**

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CREKQ YLINSQCCSL CQPGQKLVSD  
CTEFTETECL PCGESEFLDT WNRETHCHQH KYCDPNLGLR VQQKGTSETD  
TICTCEEGWH CTSEACESCV LHRSCSPGFG VKQIATGVSD TICEPCPVGF  
FSNVSSAFEK CHPWTSCKET DLVVQQAGTN KTDVVCG
```

## **[ ACTIVITY ]**

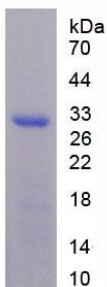
CD40, also known as Tumor Necrosis Factor Receptor Superfamily, Member 5 (TNFRSF5), is a 45-50 kDa type I transmembrane glycoprotein member of the TNF receptor superfamily. Mature human CD40 consists of a 173 amino acid (aa) extracellular domain, a transmembrane domain, and a 62 aa cytoplasmic domain. CD40 is expressed on the surface of B cells, dendritic cells, macrophages, monocytes and platelets, as well as endothelial and epithelial cells. Interaction of CD40 with its ligand, CD40 Ligand, leads to the aggregation of CD40 molecules resulting in the initiation of bidirectional intracellular signaling in both CD40 and CD40 Ligand expressing cells. A binding ELISA assay was conducted to detect the interaction of recombinant human TNFRSF5 and recombinant human TNFSF5. Briefly, TNFRSF5 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100µl were then transferred to TNFSF5-coated microtiter wells and incubated for 1h at 37°C. Wells were washed with PBST and incubated for 1h with anti-TNFRSF5 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 50µL stop solution to the wells and read at 450/630nm immediately. The binding activity of TNFRSF5 and TNFSF5 was shown in Figure 1, the ED50 for this effect is 120 ng/mL.



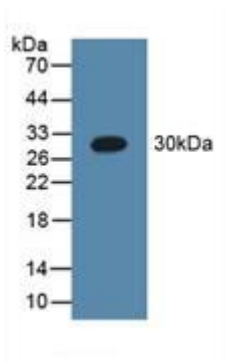
**Figure 1. The binding activity of TNFRSF5 with TNFSF5**

## [ IDENTIFICATION ]



**Figure 2. SDS-PAGE**

**Sample: Active recombinant CD40, Human**



**Figure 3. Western Blot**

**Sample: Recombinant CD40, Human;**

**Antibody: Rabbit Anti- Human CD40 Ab (PAB249Hu06)**

**[ 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.