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APA652Ra01 100µg Active Cluster Of Differentiation 28 (CD28) 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: Asn20~Leu150 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: 6.5 Predicted Molecular Mass: 18.7kDa Accurate Molecular Mass: 19kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

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.

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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.

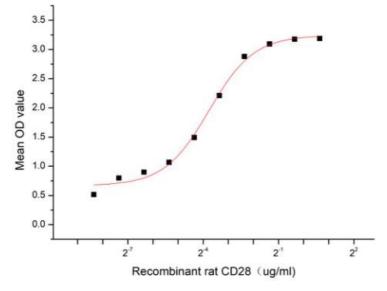
[SEQUENCE]

N KILVKQSPLL VVDNNEVSLS CRYSYNLLAK EFRASLYKGV NSDVEVCVGN GNFTYQPQFR PNVGFNCDGN FDNETVTFRL WNLDVNHTDI YFCKIEVMYP PPYLDNEKSN GTIIHIKEKH LCHAQTSPKL

[ACTIVITY]

The cluster of differentiation (CD) antigen CD28 is a member of the immunoglobulin subfamily. CD28 is a central co-stimulatory molecule for TCR-mediated activation such as cytokine production and T-cell proliferation upon ligand binding and TCR stimulation. CD80 and CD86 are expressed on antigen presenting cells and CD86 is primary ligand for CD28. Thus a functional binding ELISA assay was conducted to detect the interaction of recombinant rat CD28 and recombinant human CD86. Briefly, CD28 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ I were then transferred to CD86-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-CD28 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37 $^{\circ}$ 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 450/630 nm immediately. The binding activity of recombinant rat CD28 and recombinant human CD86 was shown in Figure 1, the EC50 for this effect is 0.07 ug/mL.

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[IDENTIFICATION]

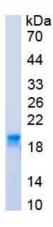


Figure 2. SDS-PAGE

Sample: Active recombinant CD28, Rat

[<u>IMPORTANT NOTE</u>]

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.