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APA300Mu01 100µg Active Neutrophil Cytosolic Factor 2 (NCF2) Organism Species: *Mus musculus (Mouse) 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: Lys354~Val525 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: 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: 23.2kDa Accurate Molecular Mass: 27kDa 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.

[<u>SEQUENCE</u>]

KVHYKYT VVMETRLGLP YSQLRNMVSK KLALSPEHTK LSYRRDSHE LLLLSEESMK DAWGQVKNYC LTLWCEHTVG DQGLIDEPIQ RENSDASKQT TEPQPKEGTQ VVAIFSYEAA QPEDLEFVEG DVILVLSHVN EEWLEGECKG KVGIFPKAFV EGCAAKNLEG IPREV

[ACTIVITY]

Neutrophil Cytosolic Factor 2 (NCF2), as kown as as NCF-2, NOXA2, P67PHOX, and P67-PHOX, is a vital component of the NADPH oxidase complex in neutrophils, essential for immune defense against pathogens. Its dysfunction can lead to severe immune disorders like CGD. It has been reported that when the cell is properly stimulated, NCF2 binds to NOX1 and other necessary subunits such as p47phox and p22phox to form a complete NADPH oxidase complex. Thus a functional ELISA assay was conducted to detect the interaction of recombinant mouse NCF2 and recombinant human NOX1. Briefly, NCF2 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 µI were then transferred to NOX1-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-NCF2 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 mouse NCF2 and recombinant human NOX1 was shown in Figure 1, the EC50 for this effect is 0.05ug/mL.

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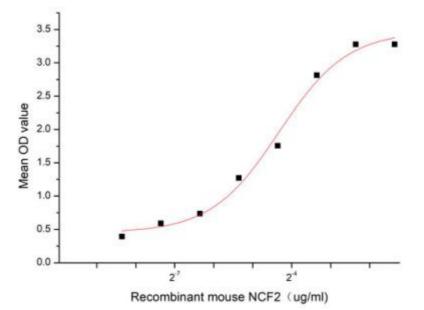


Figure 1. The binding activity of recombinant mouse NCF2 and human NOX1

[IDENTIFICATION]

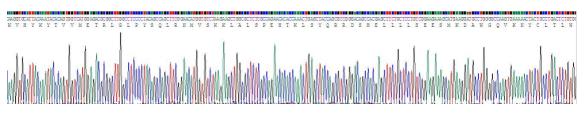


Figure 2. Gene Sequencing (extract)

	kDa 70
	44
	33
-	26
	22
	18
	14
8	10

Figure 3. SDS-PAGE

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Sample: Active recombinant NCF2, Mouse

[<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.