

APA859Hu03 100µg
Active Mannose Associated Serine Protease 2 (MASP2)
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: Ile445~Ile683

Tags: N-terminal His-tag

Purity: >98%

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

Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.

Original Concentration: 1000µg/mL

Applications: Cell culture; Activity Assays; In vivo assays.

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

Predicted isoelectric point: 5.7

Predicted Molecular Mass: 27.4kDa

Accurate Molecular Mass: 28kDa 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]

IYGGQK
AKPGDFPWQV LILGGTTAAG ALLYDNWVLT AAHAVYEQKH DASALDIRMG
TLKRLSPHYT QAWSEAVFIH EGYTHDAGFD NDIALIKLNN KVVINSNITP
ICLPRKEAES FMRTDDIGTA SGWGLTQGRF LARNLMYVDI PIVDHQKCTA
AYEKPPYPRG SVTANMLCAG LESGGKDSR GDSGGALVFL DSETERWFG
GIVSWGSMNC GEAGQYGVYT KVINYIPWIE NII

[ACTIVITY]

MASP2 (Mannan-binding lectin serine protease 2) is a serum protease that plays an important role in the activation of the complement system via mannan-binding lectin. The preproprotein of MASP2 is proteolytically processed to generate A and B chains that heterodimerize to form the mature protease, which is able to associate with MBL2. Thus, a functional binding ELISA assay was constructed to detect the association of rhMASP2 with MBL2. Briefly, rhMASP-2 were diluted serially in 10 mM Tris-HCl, 1 M NaCl, 5mM CaCl₂, and 0.05% Triton X-100(pH 7.4). Duplicate samples of 100 ul were then transferred to MBL2-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1 h with anti-MASP-2 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated for 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 MASP2 with MBL2 was shown in Figure 1 and this effect was in a dose dependent manner.

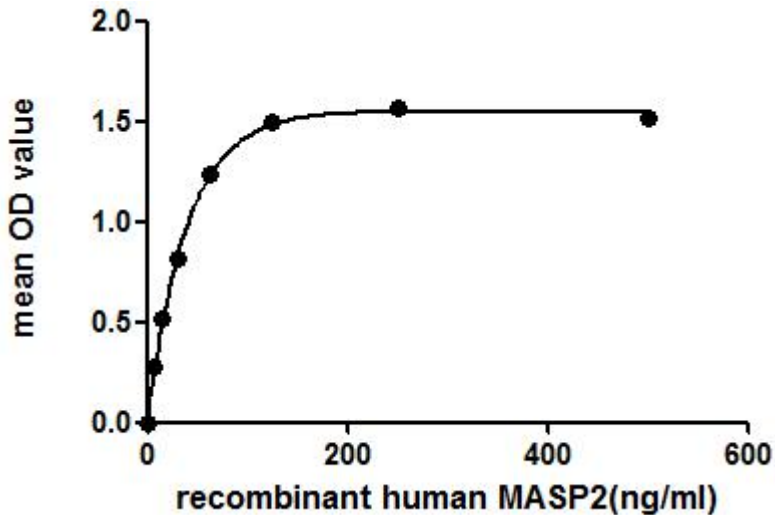


Figure 1. The binding activity of MASP2 with MBL2

The activity of MASP2 was also measured by its ability to cleaves a thioester substrate Z-Lys-SBzl•HCl. The reaction was performed in 0.05 M Tris, pH 8.5 (assay buffer), initiated by addition 50 μ L of various concentrations of MASP2 (diluted by assay buffer) to 50 μ L of substrate and DTNB mixture (equal volumes mixed by 0.4 mM substrate and 0.4 mM DTNB). The final well serves as a negative control with no MASP2, replaced with 50 μ L assay buffer. Incubated at 25 $^{\circ}$ C for 5min, then read at a wavelength of 405 nm. The specific activity of recombinant human MASP2 is >30 pmol/min/ μ g.

Specific Activity (pmol/min/ μ g)=

Adjusted V_{max} * (OD/min) x well volume (L) x 10^{12} pmol/mol

 ext. coeff** (M $^{-1}$ cm $^{-1}$) x path corr.*** (cm) x amount of enzyme (μ g)

*Adjusted for Substrate Blank

**Using the extinction coefficient 13260 M $^{-1}$ cm $^{-1}$

***Using the path correction 0.320 cm

[IDENTIFICATION]

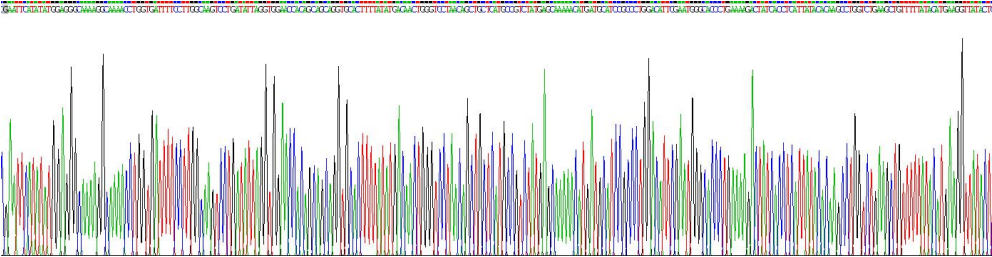


Figure 2. Gene Sequencing (extract)

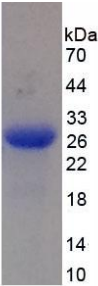


Figure3. SDS-PAGE

Sample: Active recombinant MASP2, Human

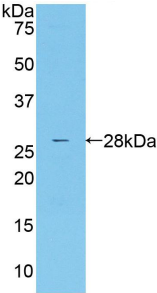


Figure 4. Western Blot

Sample: Recombinant MASP2, Human;

Antibody: Rabbit Anti-Human MASP2 Ab (PAA859Hu03)

[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.