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APB480Hu02 100µg Active Mannose Binding Lectin (MBL) Organism Species: Homo sapiens (Human) *Instruction manual*

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1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Leu130~lle248 Tags: Two N-terminal Tags, His-tag and GST-tag Purity: >98% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01% sarcosyl, 5% trehalose, and Proclin300. 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: 5.8 Predicted Molecular Mass: 43.0kDa Accurate Molecular Mass: 43kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) 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]

L GKQVGNKFFL TNGEIMTFEK VKALCVKFQA SVATPRNAAE NGAIQNLIKE EAFLGITDEK TEGQFVDLTG NRLTYTNWNE GEPNNAGSDE DCVLLLKNGQ WNDVPCSTSH LAVCEFPI

[ACTIVITY]

MBL2 (Mannose-binding protein C) is a calcium-dependent lectin involved in innate immune defense, which binds mannose, fucose and N-acetylglucosamine on different microorganisms, therefore results in activation of the lectin pathway of the complement system. It has been proven that MASP-2 (Mannan-binding lectin serine protease 2) forms complexes with the pattern recognition molecules MBL2, triggers the activation of the complement system. Thus, a functional binding ELISA assay was constructed to detect the association of rhMBL2 with MASP2. Briefly, rhMBL2 were diluted serially in 10mM Tris-HCI, 1M NaCI, 5mM CaCl₂, and 0.05%Triton X-100 (pH 7.4). Duplicate samples of 100uL were then transferred to MASP2-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-MBL2 mAb, then aspirated and washed 3 times. After incubation with HRP labeled secondary antibody, wells were incubated for 15-25 minutes at 37°C. Finally, add 50µL stop solution to the wells and read at 450nm immediately.

The binding activity of MBL2 with MASP2 was shown in Figure 1 and this effect was in a dose dependent manner.

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Figure 1. The binding activity of MBL2 with MASP2.



Figure 2. Gene Sequencing (extract)



Figure 3. SDS-PAGE Sample: Active recombinant MBL, Human

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Figure 4. Western Blot

Sample: Recombinant MBL, Human;

Antibody: Rabbit Anti-Human MBL Ab (PAB480Hu02)

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