Coud-Clone Corp.

APA480Hu01 100µg Active Myoglobin (MYO) Organism Species: *Homo sapiens (Human) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Met1~Gly154 Tags: Two N-terminal Tags, His-tag and GST-tag Purity: >95% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 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: 6.9

Predicted Molecular Mass: 47.2kDa

Accurate Molecular Mass: 47kDa 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.



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>]

MGLSDGEWQL VLNVWGKVEA DIPGHGQEVL IRLFKGHPET LEKFDKFKHL KSEDEMKASE DLKKHGATVL TALGGILKKK GHHEAEIKPL AQSHATKHKI PVKYLEFISE CIIQVLQSKH PGDFGADAQG AMNKALELFR KDMASNYKEL GFQG

[ACTIVITY]

Myoglobin (symbol Mb or MB or MYO) is an iron- and oxygen-binding protein found in the muscle tissue of vertebrates in general and in almost all mammals. It is related to hemoglobin, which is the iron- and oxygen-binding protein in blood, specifically in the red blood cells. Myoglobin is found in Type I muscle, Type II A and Type II B, but most texts consider myoglobin not to be found in smooth muscle. Besides, Proteasome 26S Subunit, Non ATPase 4 (PSMD4) has been identified as an interactor of MYO, thus a binding ELISA assay was conducted to detect the interaction of recombinant human MYO and recombinant human PSMD4 Briefly, MYO were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100µL were then transferred to PSMD4-coated microtiter wells and incubated for 2h at 37℃. Wells were washed with PBST and incubated for 1h with anti-MYO 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 15-25 minutes at 37°C. Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of MYO and PSMD4 was shown in Figure 1, and this effect was in a dose dependent manner.

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Figure 1. The binding activity of MYO with PSMD4.



Figure 2. SDS-PAGE

Sample: Active recombinant MYO, Human





Figure 3. Western Blot Sample: Recombinant MYO, Human; Antibody: Rabbit Anti-Human MYO Ab (PAA480Hu01)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.