

APG790Hu01 100µg
Active Mdm2 p53 Binding Protein Homolog (MDM2)
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~Pro218

Tags: N-terminal His-tag

Purity: >95%

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: 4.3

Predicted Molecular Mass: 39.7kDa

Accurate Molecular Mass: 44kDa as determined by SDS-PAGE reducing conditions.

Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

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.

[SEQUENCE]

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MCNTNMSVPT DGAVTTSQIP ASEQETLDYW KCTSCNEMNP PLPSHCNRCW  
ALRENWLPED KGKDKGEISE KAKLENSTQA EEGFDVPDCK KTIVNDSRES  
CVEENDDKIT QASQSQESD YSQPSTSSSI IYSSQEDVKE FERREETQDKE  
ESVESSLPLN AIEPCVICQG RPKNGCIVHG KTGHLMACFT CAKKLLKRNK  
PCPVC RQPIQ MIVLTYFP
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[ACTIVITY]

Mouse double minute 2 homolog (MDM2) also known as E3 ubiquitin-protein ligase Mdm2 is a cellular oncoprotein that recognizes the N-terminal trans-activation domain (TAD) of the p53 tumor suppressor and as an inhibitor of p53 transcriptional activation. The human homologue of this protein is sometimes called Hdm2. The p53 tumor suppressor is the key target of MDM2. It has been identified as a p53 interacting protein that represses p53 transcriptional activity and also acts as an E3 ubiquitin ligase, targeting both itself and p53 for

degradation by the proteasome. MDM2 is capable of auto-polyubiquitination, and in complex with p300, a cooperating E3 ubiquitin ligase, is capable of polyubiquitinating p53. Besides, S100 Calcium Binding Protein (S100) has been identified as an interactor of MDM2, thus a binding ELISA assay was conducted to detect the interaction of recombinant human MDM2 and recombinant human S100. Briefly, MDM2 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to S100-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-MDM2 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 of MDM2 and S100 was shown in Figure 1, and this effect was in a dose dependent manner.

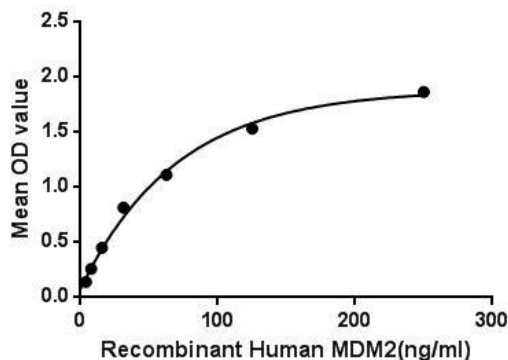


Figure 1. The binding activity of MDM2 with S100.

[IDENTIFICATION]

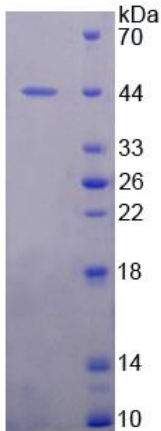


Figure 2. SDS-PAGE

Sample: Active recombinant MDM2, Human

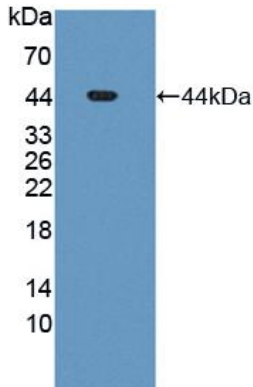


Figure 3. Western Blot

Sample: Recombinant MDM2, Human;

Antibody: Rabbit Anti-Human MDM2 Ab (PAG790Hu01)