

APA399Hu61 50µg
Active High Mobility Group Protein 1 (HMGB1)
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: Eukaryotic expression.

Host: 293F cell

Residues: Met1~Glu215

Tags: N-terminal His Tag and C-terminal Fc Region of Human IgG1

Purity: >95%

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

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 5%Trehalose.

Applications: Activity Assays.

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

Predicted isoelectric point: 5.6

Predicted Molecular Mass: 51.2kDa

Accurate Molecular Mass: 65kDa 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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MGKGDPPKPR GKMSYAFFV QTCREEHKKK HPDASVNFSE FSKKCSERWK  
TMSAKEKGF EDMAKADKAR YEREMKTYIP PKGETKKKFK DPNAPKRPPS  
AFFLFCSEYR PGIKGEHPGL SIGDVAKKLG EMWNNTAADD KQPYEKKA  
LKEKYEKDIA AYRAKGPDA AKKGVVKA EK SKKKKEEEED EDEEDEEEE  
EDEEDEEEE DDDDE
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[ACTIVITY]

High Mobility Group Protein 1 (HMG1), also known as high mobility group box 1 protein belongs to high mobility group and contains HMG-box domain. HMG1 is one of the most important chromatin proteins. This nuclear protein organizes the DNA and regulates transcription. It supports transcription of many genes in interactions with many transcription factors. HMGB1 is secreted by immune cells (like macrophages, monocytes and dendritic cells) through leaderless secretory pathway. Activated macrophages and monocytes secrete HMGB1 as a cytokine mediator of Inflammation. Besides, Tumor Protein p53 (TP53) has been identified as an interactor of HMG1, thus a binding ELISA assay was conducted to detect the interaction of recombinant human HMG1 and recombinant human TP53. Briefly, HMG1 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to TP53-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-HMG1 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 HMG1 and TP53 was shown in Figure 1, and this effect was in a dose dependent manner.

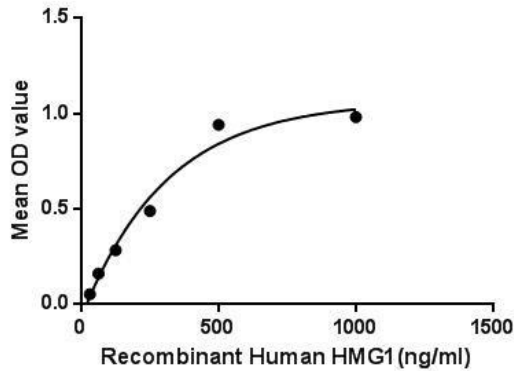


Figure 1. The binding activity of HMG1 with TP53.

[IDENTIFICATION]

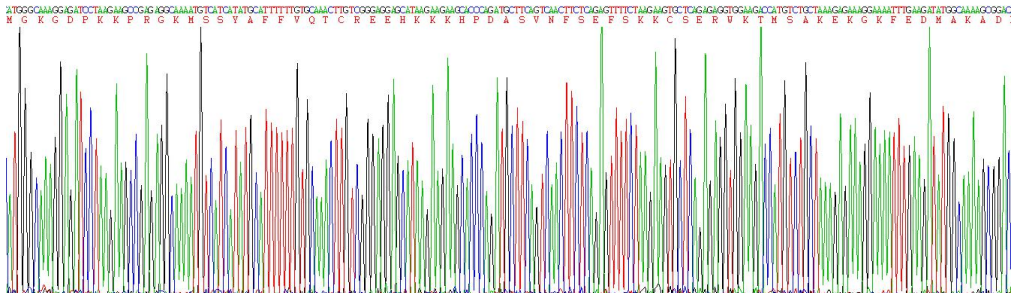


Figure 2. Gene Sequencing (extract)

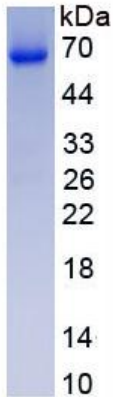


Figure 3. SDS-PAGE

Sample: Active recombinant HMGB1, Human

[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.