

APA399Ra01 100µg
Active High Mobility Group Protein 1 (HMGB1)
Organism Species: *Rattus norvegicus* (Rat)
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: Met1~Glu215

Tags: N-terminal His-tag

Purity: >90%

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

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

Original Concentration: 200µg/mL

Applications: Cell culture; Activity Assays.

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

Predicted isoelectric point: 5.9

Predicted Molecular Mass: 24.9kDa

Accurate Molecular Mass: 30kDa 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 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]

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MGKGDPPKPR  GKMSYAFFV  QTCREEHKKK  HPDASVNFSE  FSKKCSERWK  
TMSAKEKGF  EDMAKADKAR  YEREMKTYIP  PKGETKKKFK  DPNAPKRPPS  
AFFLFCSEYR  PKIKGEHPGL  SIGDVAKKLG  EMWNNTAADD  KQPYEKKA  
LKEKYEKDI  AYRAKGPDA  AKKGVVKA  SKKKKEEEDD  EEEDEEEEE  
EEEEDEEEE  DDDDE
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[ACTIVITY]

High Mobility Group Protein 1 (HMGB1) is among the most important chromatin proteins. In the nucleus HMGB1 interacts with nucleosomes, transcription factors, and histones. This nuclear protein organizes the DNA and regulates transcription. Besides, Toll Like Receptor 2 (TLR2) has been identified as an interactor of HMGB1, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant rat HMGB1 and recombinant human TLR2. Briefly, HMGB1 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 ul were then transferred to TLR2-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-HMGB1 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 50 µL stop solution to the wells and read at 450/630 nm immediately. The binding activity of recombinant rat HMGB1 and recombinant human TLR2 was shown in Figure 1, the EC50 for this effect is 0.25 µg/mL.

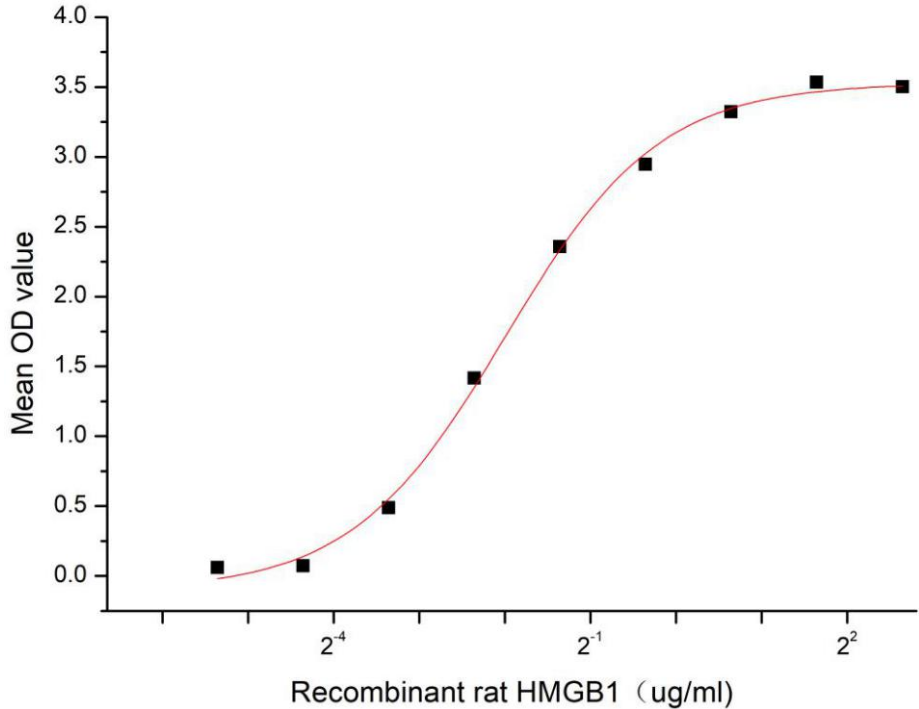


Figure 1. The binding activity of recombinant rat HMGB1 and recombinant human TLR2

[IDENTIFICATION]

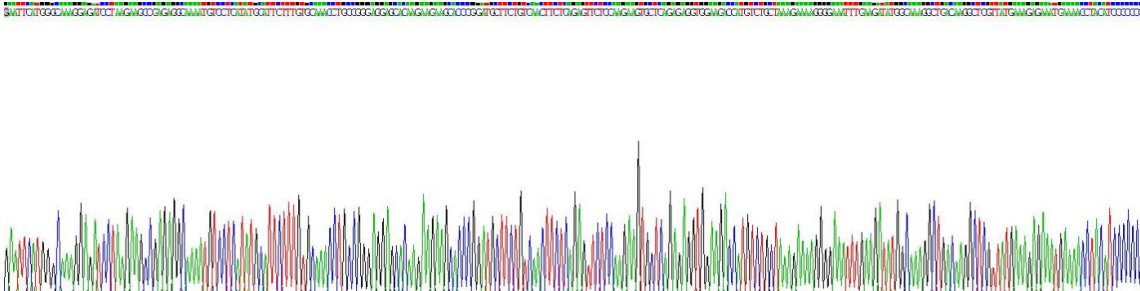


Figure 2. Gene Sequencing (extract)

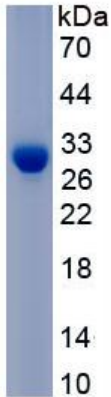


Figure 3. SDS-PAGE

Sample: Active recombinant HMGB1, Rat

[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.