

**APA399Hu64 100µg**  
**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

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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 Rabbit IgG

**Purity:** >95%

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

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

**Original Concentration:** 200µg/mL

**Applications:** Activity Assays.

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

**Predicted isoelectric point:** 5.4

**Predicted Molecular Mass:** 49.9kDa

**Accurate Molecular Mass:** 68kDa 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 ]**

MGKGDPKKPRGKMSSYAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWKTMSAKEKGKFKED  
MAKADKARYEREMKTYIPPKGETKKKFKDPNAPKRPPSAFFLCSEYRPKIKGEHPGLSIGDVAKK  
LGEMWNNTAADDKQPYEKKAALKKEYEKDIAAYRAKGKPDAAKKGVVKAESKKKKKEEEEDE  
EDEEDEEEEEDEEDEDEEEEDDDDE

## **[ ACTIVITY ]**

High Mobility Group Protein 1 (HMG1) 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, Cluster Of Differentiation 24 (CD24) 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 CD24. Briefly, biotin-linked HMG1 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl were then transferred to CD24-coated microtiter wells and incubated for 1h at 37 °C . Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 30min, then wells were aspirated and washed 5 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 recombinant human HMG1 and recombinant human CD24 was

shown in Figure 1, the EC50 for this effect is 0.16ug/mL.

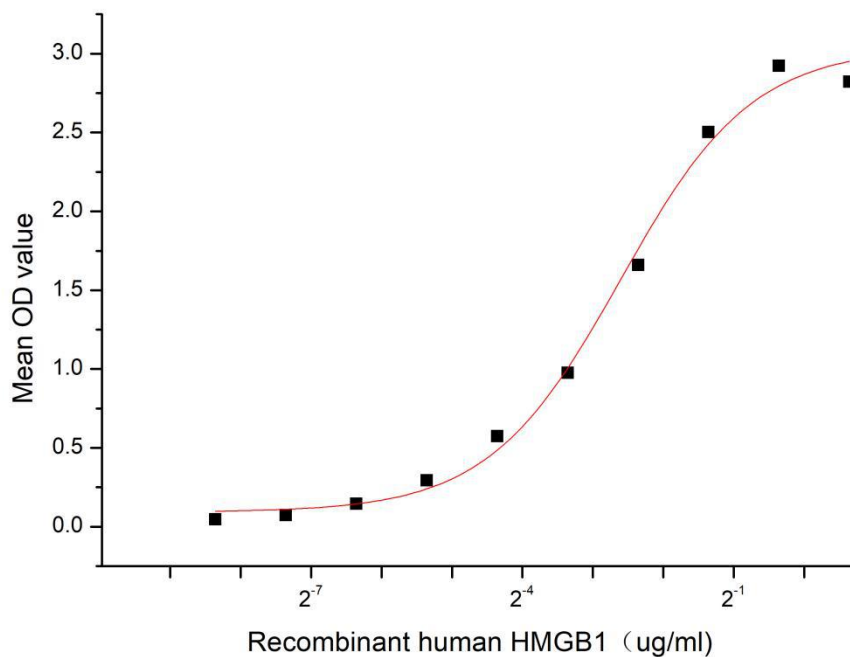


Figure 1. The binding activity of recombinant human HMGB1 and recombinant human CD24

## [ IDENTIFICATION ]

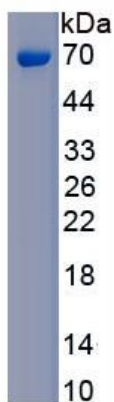


Figure 2. SDS-PAGE

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.