

Datasheet for ABIN6574156

**HMGB1 ELISA Kit**

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## Overview

Quantity:	96 tests
Target:	HMGB1
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Detection Range:	46.88 pg/mL - 3000 pg/mL
Minimum Detection Limit:	46.88 pg/mL
Application:	ELISA

## Product Details

Purpose:	The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of HMG1 in mouse serum, plasma.  We offer <b>validation data (WB) for the kit components</b> . So you can be sure to order a reliable ELISA kit product composed of high quality reagents.
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of High Mobility Group Protein 1 (HMGB1)
Cross-Reactivity (Details):	No significant cross-reactivity or interference between High Mobility Group Protein 1 (HMG1) and analogues was observed.

## Product Details

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Sensitivity: 18.29 pg/mL

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Components:

- Pre-coated, ready to use 96-well strip plate, flat bottom
- Plate sealer for 96 wells
- Reference Standard
- Standard Diluent
- Detection Reagent A
- Detection Reagent B
- Assay Diluent A
- Assay Diluent B
- Reagent Diluent (if Detection Reagent is lyophilized)
- TMB Substrate
- Stop Solution
- Wash Buffer (30 x concentrate)
- Instruction manual

## Target Details

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Target: HMGB1

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Alternative Name: High Mobility Group Protein 1 (HMGB1) ([HMGB1 Products](#))

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UniProt: [P63158](#)

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Pathways: [p53 Signaling](#), [Regulation of Muscle Cell Differentiation](#), [Skeletal Muscle Fiber Development](#), [Positive Regulation of Endopeptidase Activity](#), [Regulation of Carbohydrate Metabolic Process](#), [Toll-Like Receptors Cascades](#), [Smooth Muscle Cell Migration](#), [Inflammasome](#)

## Application Details

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Application Notes:

- Limited by the current condition and scientific technology, we cannot completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit.
- The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.
- Kits from different batches may be a little different in detection range, sensitivity and color developing time.
- Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
- Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism.
- There may be some foggy substance in the wells when the plate is opened at the first time. It

will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed.

- Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at  $450 \pm 10$ nm wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment.
- Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.
- Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too.
- Kits from different manufacturers for the same item might produce different results, since we have not compared our products with other manufacturers.

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Comment:

Information on standard material:

The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative.

Information on reagents:

The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them.

Information on antibodies:

The provided antibodies and their host vary in different kits.

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Sample Volume: 100  $\mu$ L

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Assay Time: 3 h

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Plate: Pre-coated

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Protocol:

1. Prepare all reagents, samples and standards,
2. Add 100 $\mu$ L standard or sample to each well. Incubate 1 hours at 37 °C,
3. Aspirate and add 100 $\mu$ L prepared Detection Reagent A. Incubate 1 hour at 37 °C,
4. Aspirate and wash 3 times,
5. Add 100 $\mu$ L prepared Detection Reagent B. Incubate 30 minutes at 37 °C,
6. Aspirate and wash 5 times,

7. Add 90µL Substrate Solution. Incubate 10-20 minutes at 37 °C,
8. Add 50µL Stop Solution. Read at 450nm immediately.

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### Reagent Preparation:

1. Bring all kit components and samples to room temperature (18-25 °C) before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.
2. Standard - Reconstitute the Standard with 1.0 mL of Standard Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 6,000pg/mL. Firstly dilute the stock solution to 3,000pg/mL and the diluted standard serves as the highest standard (3,000pg/mL). Then prepare 7 tubes containing 0.5 mL Standard Diluent and use the diluted standard to produce a double dilution series. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 3,000pg/mL, 1,500pg/mL, 750pg/mL, 375pg/mL, 187.5pg/mL, 93.75pg/mL, 46.88pg/mL, and the last microcentrifuge tube with Standard Diluent is the blank as 0pg/mL.
3. Detection Reagent A and Detection Reagent B - If lyophilized reconstitute the Detection Reagent A with 150µL of Reagent Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with Assay Diluent A and B, respectively.
4. Wash Solution - Dilute 20 mL of Wash Solution concentrate (30x) with 580 mL of deionized or distilled water to prepare 600 mL of Wash Solution (1x).
5. TMB substrate - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

### Note:

1. Making serial dilution in the wells directly is not permitted.
2. Prepare standards within 15 minutes before assay. Please do not dissolve the reagents at 37 °C directly.
3. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL for one pipetting.
4. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.
5. If crystals have formed in the Wash Solution concentrate (30x), warm to room temperature and mix gently until the crystals are completely dissolved.
6. Contaminated water or container for reagent preparation will influence the detection result.

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### Sample Preparation:

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturation may occur in these samples, leading to false results. Samples should therefore be stored for a short period at 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thaw cycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged to remove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to

## Application Details

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determine compatibility with the kit.

- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance. The recommended dilution factor is for reference only.
- Please estimate the concentration of the samples before performing the test. If the values are not in the range of the standard curve, the optimal sample dilution for the particular experiment has to be determined.

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Assay Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level of target were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level of target were tested on 3 different plates, 8 replicates in each plate. CV(%) = SD/meanX100 Intra-Assay: CV < 10% Inter-Assay: CV < 12%
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Restrictions:	For Research Use only
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## Handling

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Precaution of Use:	The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
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Storage:	4 °C/-20 °C
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Storage Comment:	1. For unopened kit: All reagents should be stored according to the labels on the vials. The Standard, Detection Reagent, and 96-well Strip Plate should be stored at -20 °C upon receipt, while the other reagents should be stored at 4 °C. 2. For opened kits: the remaining reagents must be stored according to the above storage conditions. In addition, please return the unused wells to the foil pouch containing the desiccant and seal the foil pouch with the zipper.
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Expiry Date:	6 months
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## Publications

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Product cited in:	Lenkiewicz, Adamiak, Thapa, Bujko, Pedziwiatr, Abdel-Latif, Kucia, Ratajczak, Ratajczak: "The Nlrp3 Inflammasome Orchestrates Mobilization of Bone Marrow-Residing Stem Cells into Peripheral Blood." in: <b>Stem cell reviews and reports</b> , Vol. 15, Issue 3, pp. 391-403, (2019) ( <a href="#">PubMed</a> ).
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Lee, Wang, Li, Liu: "Anti-inflammatory effect of cinnamaldehyde and linalool from the leaf essential oil of *Cinnamomum osmophloeum* Kanehira in endotoxin-induced mice." in: **Journal of food and drug analysis**, Vol. 26, Issue 1, pp. 211-220, (2019) ([PubMed](#)).

Park, Kim, Kim, Chang: "Luteolin activates ERK1/2- and Ca<sup>2+</sup>-dependent HO-1 induction that reduces LPS-induced HMGB1, iNOS/NO, and COX-2 expression in RAW264.7 cells and mitigates acute lung injury of endotoxin mice." in: **Inflammation research : official journal of the European Histamine Research Society ... [et al.]**, Vol. 67, Issue 5, pp. 445-453, (2018) ([PubMed](#)).

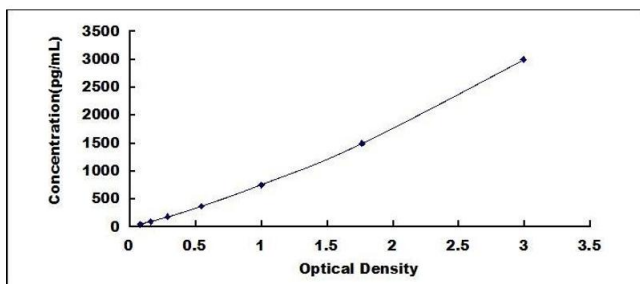
Kim, Park, Kim, Chang: "Sirt1 S-nitrosylation induces acetylation of HMGB1 in LPS-activated RAW264.7 cells and endotoxemic mice." in: **Biochemical and biophysical research communications**, Vol. 501, Issue 1, pp. 73-79, (2018) ([PubMed](#)).

Wang, Li, Deng, Liu, He: "Ursolic Acid Ameliorates Inflammation in Cerebral Ischemia and Reperfusion Injury Possibly via High Mobility Group Box 1/Toll-Like Receptor 4/NFκB Pathway." in: **Frontiers in neurology**, Vol. 9, pp. 253, (2018) ([PubMed](#)).

There are more publications referencing this product on: [Product page](#)

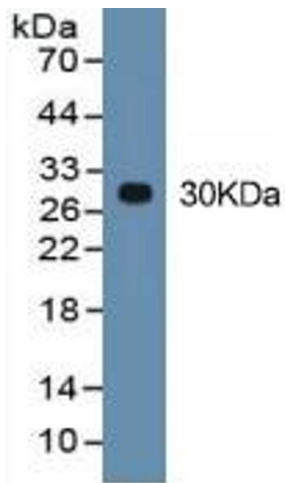
## Images

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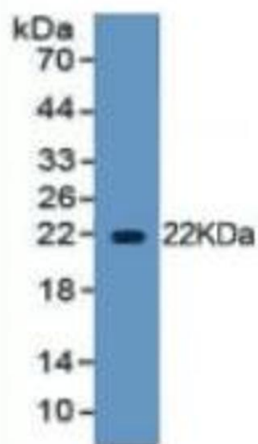
### ELISA

**Image 1.** Typical standard curve



#### Western Blotting

**Image 2.** Rabbit Capture antibody from the kit in WB with Positive Control: HepG2 cell lysate.



#### Western Blotting

**Image 3.** WB of Protein Standard: different control antibodies against Highly purified E. coli-expressed recombinant mouse HMG1.

Please check the [product details page](#) for more images. Overall 6 images are available for ABIN6574156.