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## **HMGB1 ELISA Kit**





**Publications** 



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Quantity:	96 tests	
Target:	HMGB1	
Reactivity:	Human	
Method Type:	Sandwich ELISA	
Detection Range:	78 pg/mL - 5000 pg/mL	
Minimum Detection Limit:	78 pg/mL	
Application:	ELISA	
Product Details		
Purpose:	For the quantitative determination of human high mobility group protein B1 (HMGB-1)	
	concentrations in serum, plasma, tissue homogenates, cell lysates.	
Sample Type:	Cell Lysate, Plasma, Serum, Tissue Homogenate	
Analytical Method:	Quantitative	
Detection Method:	Colorimetric	
Specificity:	This assay has high sensitivity and excellent specificity for detection of human HMGB-1. No	
	significant cross-reactivity or interference between human HMGB-1 and analogues was	
	observed. Note: Limited by current ski lls and knowledge, it is impossible for us to complete the	
	cross-reactivity detection between human HMGB-1 and all the analogues, therefore, cross	
	reaction may still exist.	
Sensitivity:	19.5 pg/mL	

## **Product Details**

Components:

- Assay plate
- Standard
- HRP-avidin (100 x concentrate)
- Biotin-antibody (100 x concentrate)
- · Sample Diluent
- · HRP-avidin Diluent
- · Biotin-antibody Diluent
- Wash Buffer (25 x concentrate)
- · TMB Substrate
- · Stop Solution
- · Adhesive Strip

# Target Details

Target:	HMGB1
Alternative Name:	high-mobility group box 1 (HMGB1 Products)
Background:	Abbreviation: HMGB1  Alias: DKFZp686A04236, HMG1, HMG3, SBP-1,  Amphoterin OTTHUMP00000018196 OTTHUMP00000190860 OTTHUMP00000200117 Sulfoglucuronyl carbohydrate binding protein high mobility group box 1 high mobility group prot
UniProt:	P09429
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**

Application Notes:	Optimal working dilution should be determined by the investigator.	
Sample Volume:	100 μL	
Assay Time:	1 - 4.5 h	
Plate:	Pre-coated	
Protocol:	1. Prepare reagents, samples and standards as instructed.	
	2. Add 100 $\mu L$ standard or sample to each well. Incubate 2 hours at 37 °C.	
	3. Remove the liquid of each well, don't wash.	
	4. Add 100 μL Biotin-antibody (1x) to each well. Incubate 1 hour at 37 °C.	
	5. Aspirate and wash 3 times.	

- 6. Add 100 µL HRP-avidin (1x) to each well. Incubate 1 hour at 37 °C
- 7. Aspirate and wash 5 times.
- 8. Add 90  $\mu$ L of TMB Substrate to each well. Incubate for 15-30 minutes at 37 °C. Protect from light.
- 9. Add 50  $\mu$ L Stop Solution to each well. Read at 450 nm within 5 minutes.

#### Reagent Preparation:

- 1. Biotin-antibody (1x) Centrifuge the vial before opening. Biotin-antibody requires a 100-fold dilution. A suggested 100-fold dilution is 10  $\mu$ L of Biotin-antibody + 990  $\mu$ L of Biotin-antibody Diluent.
- 2. HRP-avidin (1x) Centrifuge the vial before opening. HRP-avidin requires a 100-fold dilution. A suggested 100-fold dilution is 10  $\mu$ L of HRP-avidin + 990  $\mu$ L of HRP-avidin Diluent.
- 3. Wash Buffer (1x) If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 mL of Wash Buffer (1 x).
- 4. Standard Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the Standard with 1.0 mL of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 5000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250  $\mu$ L of Sample Diluent into each tube (S0-S6). Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (5000 pg/mL). Sample Diluent serves as the zero standard (0 pg/mL).

#### Note:

- Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
- Bring all reagents to room temperature (18-25 °C) before use for 30 min.
- Prepare fresh standard for each assay. Use within 4 hours and discard after use.
- Making serial dilution in the wells directly is not permitted.
- Please carefully reconstitute Standards according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10 µL for once pipetting.
- Distilled water is recommended to be used to make the preparation for reagents.
   Contaminated water or container for reagent preparation will influence the detection result.

#### Sample Preparation:

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturationmay occur in these samples, leading to false results. Samples should therefore be stored for a short periodat 2 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thawcycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged toremove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to 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 therange of the standard curve, the optimal sample dilution for the particular
experiment has to be determined. Samples should then be diluted with PBS (pH =7.0-7.2).

#### Assay Precision:

Intra-assay Precision (Precision within an assay): CV%<8% Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<10% Three samples of known concentration were tested in twenty assays to assess.

Restrictions:

For Research Use only

### Handling

Storage:

4 °C,-20 °C

Storage Comment:

Unopened kit Store at 2 - 8°C. Do not use the kit beyond the expiration date May be stored for up to 1 month at 2 - 8°C. Coated assay Try to keep it in a sealed aluminum foil bag, plate and avoid the damp. Standard May be stored for up to 1 month at 2 - 8°C. If Biotin-antibody don't make recent use, better keep it store at HRP-avidin -20°C. Biotin-antibody Diluent Opened kit HRP-avidin Diluent Sample May be stored for up to 1 month at 2 - 8°C. Diluent Wash Buffer TMB Substrate Stop Solution \*Provided this is within the expiration date of the kit.

Expiry Date:

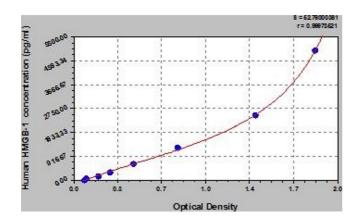
6 months

#### **Publications**

Product cited in:

Li, Zhang, Chen, Zhang, Ma, Xia: "Establishment of a rabbit model to study the influence of advanced glycation end products accumulation on osteoarthritis and the protective effect of pioglitazone." in: **Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society**, (2015) (PubMed).

Aydin, Korkusuz, Korkusuz, Tezcaner, Bilgic, Yaprakci, Keskin: "In vitro and in vivo evaluation of doxycycline-chondroitin sulfate/PCLmicrospheres for intraarticular treatment of osteoarthritis." in: **Journal of biomedical materials research. Part B, Applied biomaterials**, (2014) (PubMed).



## **ELISA**

Image 1. Typical Standard Curve