Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	105	90
	Range (%)	97-114	83-97
1:4	Average% of Expected	110	93
	Range (%)	101-117	82-101
1:8	Average% of Expected	107	95
	Range (%)	95—116	94—111
1:16	Average% of Expected	109	94
	Range (%)	98—119	87—108

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Mouse HMGB1 Immunoassay

Catalog Number: SEKM-0145

For the quantitative determination of mouse HMGB1 concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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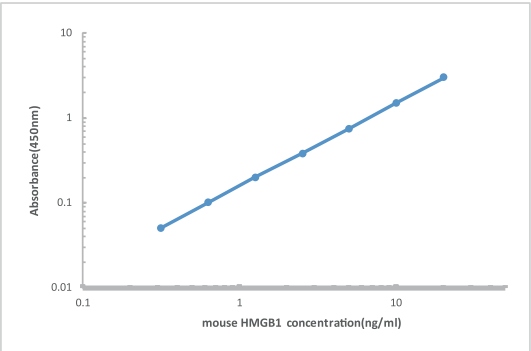
REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
RECOVERY: The recovery of HMGB1 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of HMGB1 in two matrices

Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	91	85—97
Cell culture supernatants	105	91—118

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of HMGB1 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)



Representative standard curve for HMGB1 ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 0.15ng/mL.

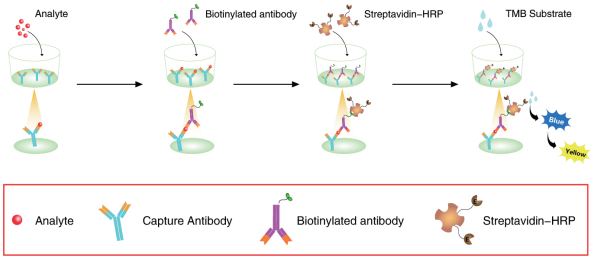
SPECIFICITY: 101% homology with human、rat、cattle and pigs

BACKGROUND

High mobility group box 1 protein, also known as high-mobility group protein 1 (HMG-1) and amphoterin, is a member of the high mobility group box family of non-histone chromosomal proteins. HMGB1 is an intracellular protein that can translocate to the nucleus where it binds DNA and regulates gene expression. HMGB1 supports transcription of many genes in interactions with many transcription factors. It also interacts with nucleosomes to loosen packed DNA and remodel the chromatin. Contact with core histones changes the structure of nucleosomes. It can also be released from cells, in which extracellular form it can bind the inflammatory receptor RAGE. Release from cells seems to involve two distinct processes: necrosis, in which case cell membranes are permeabilized and intracellular constituents may diffuse out of the cell; and some form of active or facilitated secretion induced by signaling through the NFkappaB.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for HMGB1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any HMGB1 present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for HMGB1 is added to detect the captured HMGB1 protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of Samples.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the HMGB1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the HMGB1 ELISA

Standared(ng/ml)	OD.	OD.	Average	Corrected
0	0.061	0.060	0.061	-
0.31	0.191	0.192	0.192	0.131
0.6	0.220	0.222	0.221	0.161
1.3	0.328	0.330	0.329	0.268
3	0.520	0.524	0.522	0.462
5	0.845	0.851	0.848	0.788
10	1.376	1.386	1.381	1.320
20	2.241	2.258	2.250	2.189

ASSAY PROCEDURE

Prepare all reagents and standards as directed.

⇩

Add 50μl standard or samples to each well, and add 50μl Standard/ Sample Diluent. Add 100μl working solution of Detection antibody to each well at the same time, Incubate overnight(16-18h) at 4 ° C after sealing.

⇩ Aspirate and wash 4 times

Add 100μl Substrate solution to each well, incubate 5-30 minutes, room temperature(25±2℃).

⇩

Add 50μl Stop solution to each well. Read at 450nm within 5 minutes.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard -lyophilized,100ng/ml upon reconstitution	2 vial	Aliquot and Store at -20°C** for six months
Concentrated Detection antibody(200X) - 60 ul/vial	1 vial	Store at 2-8°C **for six months
Standard Reconstitution buffer- 1 ml/vial	1 vial	Store at 2-8°C **for six months
Sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Detection antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

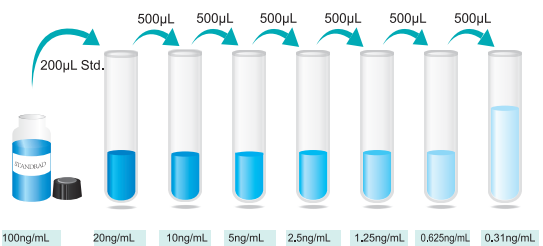
SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning:** Bring all kit components and Sample to room temperature (20-25℃) before use.
2. **Wash Buffer-** Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard/Sample -** Reconstitute the Standard with 1.0mL of Standard Reconstitution buffer. This reconstitution produces a stock solution of 100 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 800μL of Sample Diluent into the 20ng/mL tube, and add 200μL stock solution of 100ng/mL into it to get the high standard of 20ng/mL. Pipette 500μL of Sample Diluent into the remaining tubes. Use the high standard to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 20 ng/mL standard serves as the high standard. The Sample Diluent serves as the zero standard (0 ng/mL).
***If you do not run out of re-melting standard, store it at -20℃. Diluted**



Preparation of HMGB1 standard dilution

standard shall not be reused.
4. **Working solution of Detection antibody:** Make a 1:200 dilution of the solution with the Detection antibody dilution in a clean plastic tube.

*The working solution should be used within one day after dilution.