HMGB1 ELISA Kit

Enzyme Immunoassay kit for the quantification of Human/Mouse/Rat HMGB1 in serum samples

Catalog number: ARG81351

Package: 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan (R.O.C.)

Phone: +886 (3) 621 8100

Fax: +886 (3) 553 0266

Email: info@arigobio.com

INTRODUCTION

Multifunctional redox sensitive protein with various roles in different cellular compartments. In the nucleus is one of the major chromatin-associated non-histone proteins and acts as a DNA chaperone involved in replication, transcription, chromatin remodeling, V(D)J recombination, DNA repair and genome stability. Proposed to be a universal biosensor for nucleic acids. Promotes host inflammatory response to sterile and infectious signals and is involved in the coordination and integration of innate and adaptive immune responses. In the cytoplasm functions as sensor and/or chaperone for immunogenic nucleic acids implicating the activation of TLR9-mediated immune responses, and mediates autophagy. Acts as danger associated molecular pattern (DAMP) molecule that amplifies immune responses during tissue injury. Released to the extracellular environment can bind DNA, nucleosomes, IL-1 beta, CXCL12, AGER isoform 2/sRAGE, lipopolysaccharide (LPS) and lipoteichoic acid (LTA), and activates cells through engagement of multiple surface receptors. In the extracellular compartment fully reduced HMGB1 (released by necrosis) acts as a chemokine, disulfide HMGB1 (actively secreted) as a cytokine, and sulfonyl HMGB1 (released from apoptotic cells) promotes immunological tolerance. [provided by Uniprot]

PRINCIPLE OF THE ASSAY

This assay employs the sandwich enzyme immunoassay technique for the detection of Human/Mouse/Rat HMGB1 in serum samples. An antibody specific for HMGB1 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any HMGB1 present is bound on the

plate. After washing away any unbound substances, a Horseradish Peroxidase (HRP) conjugated primary antibody binds to HMGB1 is added to each well and incubates. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of total HMGB1 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of total HMGB1 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
antibody-coated microplate	12 X 8 strips	4°C
Standard (100 ng)	1 vial	4°C, aliquot & store at ≤ -20 °C after reconstitution
Standard reconstitution buffer	1.1 ml (ready-to-use)	4°C
HRP-antibody Conjugate (200X)	65 µl	4°C
Antibody diluent buffer	12 ml (ready-to-use)	4°C
Assay Buffer	6 ml (ready-to-use)	4°C
10X Wash buffer	20 ml	4°C
TMB substrate	12 ml (ready-to-use)	4°C (Protect from light)
STOP solution	6 ml (ready-to-use)	4°C
Plate sealer	1 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 1X PBS
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved. 1X Wash buffer should be prepared and stored at 4°C before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.
- Serum from various autoimmune diseases animals might contain HMGB1 autoantibodies which might result in interference of this assay.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Avoid using haemolytic, icteric or lipaemic samples and samples contain azide cannot be assayed.

REAGENT PREPARATION

- Standard: Reconstitute the standard with 1000 µl of Standard reconstitution buffer to yield a stock concentration of 100 ng/ml. Mix the standard by inversion or brief vortex for at least 5-10 seconds (do not vortex at high-speed) and stand for 10 minutes at RT to completely dissolve contents. Aliquot and store at ≤ -20°C for up to 2 weeks (store at -80°C is recommended, freeze-thaw the reconstituted standard for only once).

  Make sure the standard is dissolved completely before making serial dilutions. The 1X PBS serves as zero standard (0 ng/ml), and the standard stock can be diluted with 1X PBS as according to the suggested concentration below: 20 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml and 0.625 ng/ml.

Dilute HMGB1 standard as according to the table below:

Standard	HMGB1 Conc. (ng/ml)	µl of 1X PBS	µl of standard
S6	20	400	100 (100 ng/ml Stock)
S5	10	250	250 (S6)
S4	5	250	250 (S5)
S3	2.5	250	250 (S4)
S2	1.25	250	250 (S3)
S1	0.625	250	250 (S2)
S0	0	250	0

- 1X Wash buffer: Dilute 10X wash buffer with distilled water to yield 1X wash buffer. The diluted Wash buffer should be stored at 4 °C.
- HRP-antibody Conjugate: Diluent 200X HRP-antibody Conjugate into Antibody diluent buffer to yield 1X Detection antibody solution. The 1XHRP-antibody Conjugate solution should be used immediately.
- Sample:

  - If the sample volume is less than 50 µl, add 1X PBS to a final volume of 50 µl, for the calculation of the concentrations this dilution factor has to be taken into account.

  - If the initial assay found samples contain HMGB1 higher than the highest standard, the samples can be diluted with 1X PBS and re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  Example:

  a) Dilution 1:10: 10 µl sample + 90 µl 1X PBS (mix thoroughly).

  b) Dilution 1:100: 10 µl 1:10 diluted a) + 90 µl 1X PBS (mix thoroughly).

  Note: it is recommended to do pre-test to determine the suitable dilution factor.

ASSAY PROCEDURE

All materials except 1X wash buffer should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

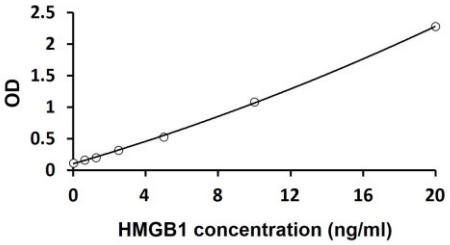
1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl** of **standards, samples and zero controls** into appropriate wells.
3. Add **50 µl** of **Assay buffer** into all wells immediately. Mix thoroughly by gently shaking or tapping the plate for few seconds. Cover the plate and **incubate for overnight (~16 hours is recommended) at 4°C** (without shaking).
4. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1X cold wash buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µl** of **1X HRP-antibody conjugate** into each well. Mix thoroughly by gently shaking or tapping the plate. Cover wells and incubate for **1 hour at 37°C.**
6. Aspirate each well and wash as step 4.
7. Add **100 µl** of **TMB substrate** to each well. Incubate for **10-15 minutes at RT** in dark. Substrate will change from colorless to different strengths of blue.
8. Add **50 µl** of **Stop solution** to each well. The color of the solution should change from blue to yellow. Mix thoroughly by gently shaking the plate.
9. Read the OD with a microplate reader at **450 nm** immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted or concentrated, the concentration read from the standard curve must be further converted by the appropriate dilution or concentration factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

Standard range: 0.625 – 20 ng/ml

Minimum Detectable Concentration: 0.4 ng/ml

Precision:

Intra-assay: 5.2 %

Inter-assay: 7.6%

Recovery:

94.8-103%

Specificity

This assay recognizes natural and recombinant total human/Mouse/Rat HMGB1.

No significant cross-reactivity or interference with recombinant human HMGB2 was observed.