

# HMGB1 ELISA Kit - for research use only -

## INTRODUCTION

High mobility group box protein 1 (HMGB1), which was originally known as amphoterin, mediates neurite outgrowth<sup>1</sup> and binds receptors for advanced glycation end products (RAGE)<sup>2</sup>. HMGB1 has 219 residues in its primary amino acid sequence, and there is > 98% sequence identity between the HMGB1 of rodents and that of humans<sup>3</sup>. In most cells, HMGB1 is located in the nucleus. It is a chromatin-associated nuclear protein that plays an important role in transcription and DNA recombination<sup>4</sup>.

Recently, HMGB1 has been shown to play a critical role in several inflammatory diseases such as septic shock<sup>5</sup>, acute lung injury<sup>6</sup> and rheumatoid arthritis<sup>7</sup>.

This kit measures only HMGB1, without measuring HMGB2 which is highly conserved (> 80% amino acid identity)<sup>8</sup>.

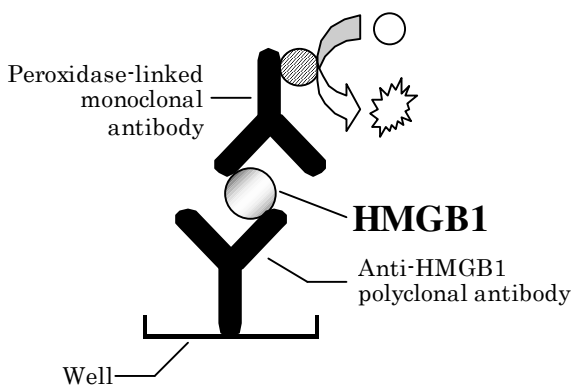
## INTENDED USE

The HMGB1 ELISA Kit is intended for the quantitative determination of HMGB1 in human, bovine, pig, rabbit, mouse and rat serum and plasma, and cell culture medium.

**This kit has been configured for research use only, and not for diagnostic or therapeutic use.**

## PRINCIPLE OF METHOD

The HMGB1 ELISA Kit is a 2-step sandwich ELISA. The wells of the microtiter strips are coated with a polyclonal antibody specific for HMGB1, with the exception of wells A and H. Diluted samples are added to the wells. During the first incubation, the HMGB1 antigen binds to the immobilized antibody. After washing, a peroxidase-linked monoclonal antibody is added. After a second incubation and washing, substrate solution is added. The color intensity is directly proportional to the HMGB1 concentration in the sample.



## KIT COMPONENTS

- |  |                      |
|--|----------------------|
| <b>1. 8-Well × 12 strips (antibody-coated)</b>               | <b>8-well</b>        |
| Anti-HMGB1 polyclonal antibody                               | × 12 strips          |
| <b>2. Standard (lyophilized)</b>                             | <b>for 1 mL × 1</b>  |
| Pig HMGB1  |                      |
| <b>3. Positive control (lyophilized)</b>                     | <b>for 1 mL × 1</b>  |
| Pig HMGB1  |                      |
| <b>4. Sample diluent solution</b>                            | <b>20 mL × 1</b>     |
| Buffer containing additives and preservative                 |                      |
| <b>5. Peroxidase-linked antibody (lyophilized)</b>           | <b>for 10 mL × 1</b> |
| Peroxidase-linked anti-HMGB1,2 monoclonal antibody           |                      |
| <b>6. Peroxidase-linked antibody dissolvent solution</b>     | <b>10 mL × 1</b>     |
| Buffer containing additives and preservative                 |                      |
| <b>7. Substrate solution A</b>                               | <b>5 mL × 1</b>      |
| 3,3', 5,5'-Tetramethyl-benzidine, dihydrochloride, dihydrate |                      |
| <b>8. Substrate solution B</b>                               | <b>5 mL × 1</b>      |
| Buffer containing 0.005 mol/L hydrogen peroxide              |                      |
| <b>9. Stop solution</b>                                      | <b>10 mL × 1</b>     |
| 0.35 mol/L sulfuric acid                                     |                      |
| <b>10. Wash solution (5x)</b>                                | <b>100 mL × 2</b>    |
| 5-Fold concentrated buffer containing Tween <sup>®</sup> 20  |                      |
| <b>11. Plate seal</b>  | <b>× 2</b>           |

## REAGENT STORAGE AND STABILITY

All kit reagents are stable until the expiration dates shown on the labels when stored at 2-8°C.

- **Microtiter plate:** Repack unused strips in the aluminum bag with desiccant and store at 2-8°C.
- **Standard, positive control and peroxidase-linked antibody:** After dissolution, aliquots are stable for one month at -30°C.

## MATERIALS REQUIRED

### BUT NOT PROVIDED

- Micropipettes and tips
- Test tubes
- Graduated cylinder
- Mixer for mixing samples and standards in test tubes (e.g. vortex mixer)
- Incubator at 37°C (also possible to use CO<sub>2</sub> incubator at 37°C)
- Incubator at 25°C (also possible at room temperature)
- Microplate washer
- Stopwatch
- Plastic wrap or plate cover
- 96-Well microplate reader capable of measurement at 450 nm
- Distilled water
- Lint-free paper towel

## REAGENT PREPARATION

### - Reconstitution and dilution of standards

Dissolve a vial of standard in 1 mL of sample diluent solution. Mix gently and allow to stand for 10 minutes to ensure complete reconstitution. Add 100  $\mu\text{L}$  of reconstituted standard to a test tube containing 700  $\mu\text{L}$  of sample diluent solution (S1: 116.8 ng/mL). Add 400  $\mu\text{L}$  of S1 to a test tube containing 400  $\mu\text{L}$  of sample diluent solution (S2: 58.4 ng/mL).

### - Reconstitution of positive control

Dissolve a vial of positive control in 1 mL of sample diluent solution. Mix gently and allow to stand for 10 minutes to ensure complete reconstitution.

### - Reconstitution of peroxidase-linked antibody

Dissolve a vial of peroxidase-linked antibody in a bottle of peroxidase-linked antibody dissolvent solution. Mix gently and allow to stand for 10 minutes to ensure complete reconstitution.

### - Substrate solution

Before use, mix the same volumes of substrate solution A and B warmed to room temperature. Use the mixed substrate solution within 30 minutes.

### - Wash buffer

Dilute 200 mL of wash solution (5x) with 800 mL of distilled water.

## SAMPLE HANDLING

In a test tube, dilute 200  $\mu\text{L}$  of fresh serum or plasma with 200  $\mu\text{L}$  of sample diluent solution. Mix the test tube contents with a mixer.

## PROCEDURAL NOTES

1. Each reagent should be warmed to room temperature at least 30 minutes before use.
2. Do not use wells A and H in the microtiter plate. They are not coated with anti-HMGB1 antibody (see Fig. 1).
3. The microtiter plate can be used without washing even if white powder is attached to the wells of the microtiter plate before use.
4. Sample diluent solution and peroxidase-linked antibody dissolvent solution can be used even if they have become cloudy. If cloudy, use them after carrying out centrifugal separation or filtration.
5. Substrate solution A and B should be warmed to room temperature before use. The prepared substrate solution must be used within 30 minutes.

## ASSAY PROCEDURE

**Note: Do not use wells A and H in microtiter plate**

1. Add 100  $\mu\text{L}$  of sample diluent buffer to the zero well (e.g.  $\blacktriangle$  in Fig. 1).
2. Add 100  $\mu\text{L}$  of diluted standards (see REAGENT PREPARATION) and positive control solution to the wells (e.g.  $\bullet$  in Fig. 1).
3. Add 100  $\mu\text{L}$  of diluted samples to the wells (e.g.  $\circ$  in Fig. 1).

	1	2	3	4	5	6	7	8	9	10	11	12
A	x	x	x	x	x	x	x	x	x	x	x	x
B	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\blacktriangle$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$
C	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\bullet$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$
D	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\bullet$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$
E	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\bullet$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$
F	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$
G	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$	$\circ$
H	x	x	x	x	x	x	x	x	x	x	x	x

x : Unusable wells.  $\circ$  : Sample wells.  
 $\blacktriangle$  : Zero well (sample diluent solution only).  
 $\bullet$  : Diluted standard (S1 and S2) and positive control

**Fig. 1: Example of use of microtiter plate**

4. Seal the plate with a plate seal and incubate at 37°C for 20-24 hours.
5. Wash wells 5 times with wash buffer using an automated or manual plate washer. After the final wash, firmly tap the plate on a lint-free paper towel to remove any remaining wash buffer.
6. Add 100  $\mu\text{L}$  of peroxidase-linked antibody solution to each well, except for wells A and H.
7. Seal the plate with a plate seal and incubate at 25°C or room temperature for 2 hours.
8. Wash wells 5 times with wash buffer using an automated or manual plate washer. After the final wash, firmly tap the plate on a lint-free paper towel to remove any remaining wash buffer.
9. Gently remove dirt from the reverse side of wells.
10. Add 100  $\mu\text{L}$  of substrate solution to each well, except for wells A and H.
11. Cover the microtiter plate with plastic wrap or a plate cover and incubate at room temperature for 30 minutes.
12. Add 100  $\mu\text{L}$  of stop solution to each well in the same sequence and at the same time intervals as the additions of substrate solution, except for wells A and H.
13. Immediately read the absorbance of the microtiter plate at 450 nm with a microplate reader.

### HMGB1 ASSAY SUMMARY

Dilute standards and samples



Add 100  $\mu\text{L}$  of diluent standards and samples to the wells

↓ Incubate for

20-24 hours at 37°C

Wash wells 5 times



Add 100  $\mu\text{L}$  of peroxidase-linked antibody solution to the wells

↓ Incubate for 2 hours at 25°C

Wash wells 5 times



Add 100  $\mu\text{L}$  of substrate solution to the wells

↓ Incubate for 30 minutes

at room temperature

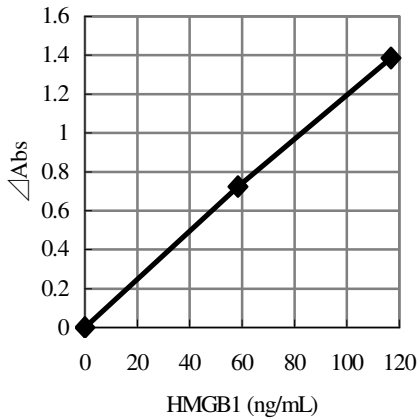
Add 100  $\mu\text{L}$  of stop solution to the wells



Read at 450 nm

## CALCULATION OF RESULTS

1. Subtract absorbance of the zero well from the absorbance of each well.
2. Plot the standards' absorbance versus the standards' concentration. Draw the best smooth curve through these points to construct the standard curve (see **Fig. 2**).
3. Read the HMGB1 concentrations of unknown samples from the standard curve. At this time, it is not necessary to take the dilution of samples into consideration.



**Fig. 2: Typical standard curve**

## ADDITIONAL INFORMATION

### - Specificity

Cross-reaction with HMGB2 is below the lower limit of detection.

### - Recovery

Recovery of serum and plasma samples is 80%-120%.

### - Reproducibility

Within-assay coefficient of variation is < 5%.

Between-assay coefficient of variation is < 8%.

### - Sensitivity

The minimum detectable concentration of HMGB1 is < 1 ng/mL.

## NOTES

1. This kit is for research use only. Do not use for diagnostic or therapeutic use.
2. Read this package insert and the latest MSDS before use.
3. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
4. Each reagent should be warmed to room temperature at least 30 minutes before use.
5. Do not use wells A and H in the microtiter plate. They are not coated with anti-HMGB1 antibody.
6. Keep strictly to the methods of reagent preparation.
7. This kit uses break-apart microtiter strips. Repack unused strips in the aluminum bag with desiccant and store at 2-8°C.
8. Remove serum or plasma from the tube immediately after centrifugation. Do not keep them for a long time in a tube because HMGB1 may be secreted due to cytolysis.
9. Sample diluent solution and peroxidase-linked antibody dissolvent solution can be used even if they have become cloudy. If cloudy, use them after carrying out centrifugal

separation or filtration.

10. Dissolved standard (before dilution), positive control and peroxidase-linked antibody should be frozen, but avoid multiple freeze-thaw cycles of frozen samples.
11. Run a separate standard curve for each assay.
12. Sample diluent solution contains a small amount of sodium azide. Sodium azide may produce an explosive azide metal if contaminated with Pb or Cu. Wash away with a large volume of water to prevent azide accumulation upon disposal.
13. The stop solution is acid (below pH 2). Therefore, care should be taken to avoid contact with skin and eyes.
14. All reagents and samples should be considered as potentially hazardous. Take care in handling and disposal. In the case of contact with skin or mucosa, wash immediately with a large volume of water.

## REFERENCES

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8. Yamada S *et al.* High mobility group protein 1 (HMGB1) quantified by ELISA with a monoclonal antibody that does not cross-react with HMGB2. *Clin Chem* 2003; **49**: 1535-1537.

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## Shino-Test Corporation

2-29-14, Oonodai, Sagamihara-shi, Kanagawa 229-0011, Japan

Phone: +81-42-753-0354

Fax: +81-42-786-8553

E-mail: HMGB\_info@shino-test.co.jp