Human HMGB1 One-Step ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: SE50009

Size: 96T

Sensitivity: 0.05 ng/mL

Range: 0.156-10 ng/mL

Usage: For the quantitative detection of human HMGB1 concentrations in serum and plasma.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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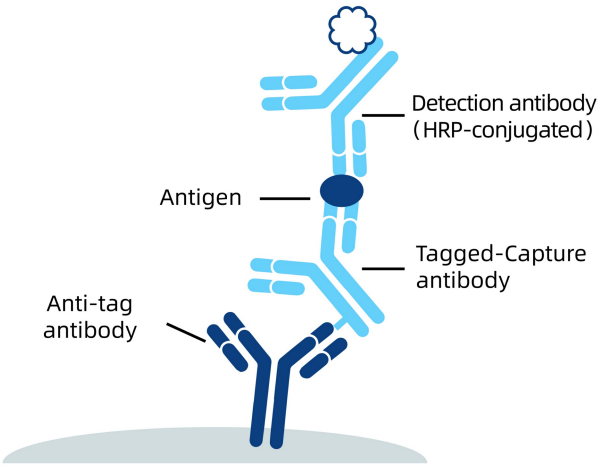
1. Background

The HMG (high mobility group) proteins are nonhistone chromosomal proteins that is present in almost all eukaryotic cells, and it functions to stabilize NUCLEOSOME formation and acts as a transcription-factor-like protein that regulates the expression of several genes. Once injury, infection or other inflammatory stimuli, activated macrophages, mature dendritic cells and natural killer cells can secret HMGB1, which act as a crucial cytokine. HMGB1 also involved in V(D)J recombination by acting as a cofactor of the RAG complex, stimulating cleavage and RAG protein binding at the 23 bp spacer of conserved recombination signal sequences (RSS). Act as a Heparin-binding protein that has a role in the extension of neurite-type cytoplasmic processes in developing cells. HMGB1 (high mobility group box 1) modulates gene expression in the nucleus, but certain immune cells secrete HMGB1 as an extracellular Alarmin to signal tissue damage.The nuclear HMGB1 relocalizes to the extracellular milieu in senescent human and mouse cells in culture and in vivo, which stimulated cytokine secretion through TLR-4 signaling.

2. Principle



An anti-tag antibody is pre-coated onto the bottom of wells. After adding antigen or samples, Tagged-Capture antibody and HRP-conjugated detection antibody, a sandwich complex is formed in the solution. TMB acts as a HRP substrate, and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns the solution yellow. The color intensity is proportional to the quantity of bound protein, which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended. Proteintech data analysis website, https: //www.ptgcn.com/products/elisa-kits/.

3.8 Microplate thermostatic shaker.

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4. Kit Components and Storage

Microplate - 96 well microplate precoated an anti-tag antibody(8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 40 ng/bottle; lyophilized	2 bottles	
Capture antibody (100X) - 60 µL/vial*	1 vial	
Detection antibody, HRP-conjugated (100X) - 60 µL/vial*	1 vial	
Sample Diluent PT 3B1 - 30 mL/bottle	1 bottle	
Detection Diluent - 15 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

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7. Regent Preparation

7.1 Wash Buffer (1×): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20×) to 570 mL deionized or distilled water to prepare 1× Wash Buffer.

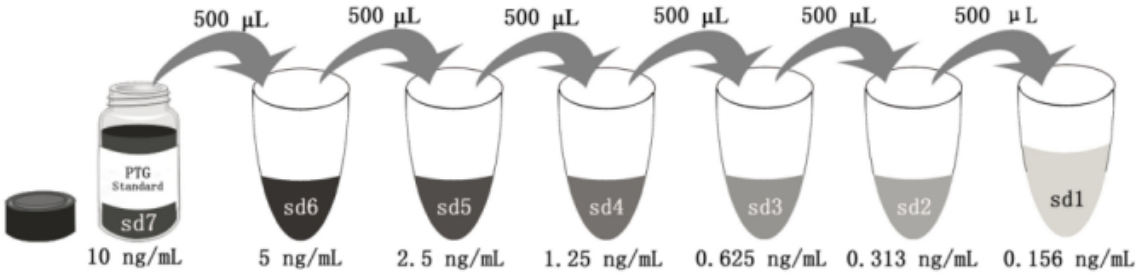
7.2 Antibody Cocktail (1×): Dilute 100× capture antibody and 100× HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 µL 100× capture antibody + 50 µL 100× Detection Antibody, HRP-conjugated + 4,900 µL Detection Diluent. Mix gently but thoroughly.

7.3 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement .

Recommended Dilution for different sample types: 1:2 or 1:4 is recommended for human serum and plasma.

7.4 Standard Serial Dilution:

Add 4 mL Sample Diluent PT 3B1 in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 3B1	**4000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated antibody can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Preset the layout of the microplate, including control group, standard group and sample group, take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Add 50 µL standard or sample to appropriate wells. To avoid high background always add samples or standards to the well before the addition of antibody cocktail.

8.3 Add 50 µL 1× Antibody Cocktail solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 1 hour (incubate at 37°C for 2 hours is recommended if thermostatic shaker is not available) .

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1× Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

8.6 Add 100 µL Stop Solution to each well in the same order as addition of the TMB substrate. Note: Avoid skin and eye contact with the Stop solution.

8.7 Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.8 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, four-parameter logistic curve- fit (4-PL) analysis is recommend. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

Procedure summary

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Add 50 µL standard or sample to appropriate wells

↓

Add 50 µL antibody cocktail solution (1x) to each well

↓

Incubate at 37 °C on a microplate thermostatic shaker set at 400 rpm for 1 hour

↓

Aspirate and wash each well four times with 400µL 1 x Wash Buffer

↓

Add 100 µL TMB substrate solution to each well

↓

Incubate at 37 °C on a microplate thermostatic shaker set at 400 rpm for 15minutes

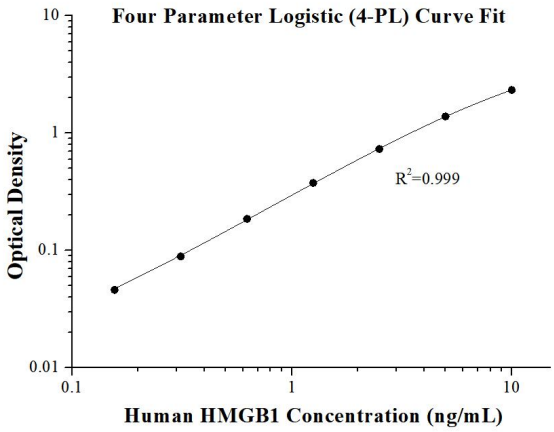
↓

Add 100 µL Stop Solution to each well and Read OD immediately

9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D	Average	Corrected
0	0.044 0.048	0.0420	-
0.156	0.0894 0.0879	0.08865	0.0463
0.3125	0.1353 0.1279	0.1316	0.0892
0.625	0.2318 0.2243	0.22805	0.1857
1.25	0.4231 0.4137	0.4184	0.376
2.5	0.7867 0.7612	0.774	0.73155
5	1.4416 1.4164	1.429	1.3866
10	2.3673 2.3809	2.3741	2.3317

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9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	8	4.71	0.10	2.21
2	8	1.28	0.05	4.28
3	8	0.66	0.03	4.90

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	16	4.66	0.21	4.40
2	16	1.25	0.05	3.89
3	16	0.66	0.03	4.85

9.3 Recovery

The recovery of human HMGB1 spiked to three different levels throughout the range of the assay in human serum was evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:2	92	75-111
	1:4	118	109-128

9.4 Sample values

Human serum - human serum samples were evaluated for the presence of human HMGB1 in this assay.

Sample Type	Mean (ng/mL)	% Detectable	Range (ng/mL)
Human serum (n=8)	3.54	75%	ND-26.74

ND*=Non-detectable

9.5 Sensitivity

The minimum detectable dose of human HMGB1 is 0.05 ng/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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9.6 Linearity

To assess the linearity of the assay, human serum samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human serum
1:2	Average% of Expected	100
	Range (%)	-
1:4	Average% of Expected	118
	Range (%)	115-122
1:8	Average% of Expected	88
	Range (%)	73-103
1:16	Average% of Expected	80
	Range (%)	79-81

9.7 Specificity

This kit specifically recognizes native and recombinant human HMGB1.

10. References

1. Yu, Miao et al. Nucleic acids research vol. 36,4 (2008): 1209-19.

2. Luan, Zheng-Gang et al. Immunobiology vol. 215,12 (2010): 956-62.

3. Lange, Sabine S, and Karen M Vasquez. Molecular carcinogenesis vol. 48,7 (2009): 571-80.

4. Davalos, Albert R et al. The Journal of cell biology vol. 201,4 (2013): 613-29.

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