Version v1b Last updated 12 August 2024

ab238543 Methylglyoxal (MG) ELISA Kit

For the quantitative measurement of Methylglyoxal adducts in protein samples such as purified protein, plasma, serum and cell lysate.

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1. Overview

Methylglyoxal ELISA Kit (ab238543) is developed for rapid detection and quantitation of MG-H1 (methyl-glyoxal-hydro-imidazolone) protein adducts.

The quantity of MG adduct in protein samples is determined by comparing its absorbance with that of a known MG-BSA standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown protein samples.

2. Protocol Summary

Prepare all reagents, samples and standards as instructed.

Add 50 µL standard or sample to wells of Methylglyoxal Conjugate coated plate and incubate for 10 minutes. Add 50 µL of the diluted anti-Methylglyoxal antibody and incubate for 1 hour.

Washing steps with 250 µL 1X Wash Buffer.

Add 100 µL diluted Secondary Antibody-HRP Conjugate per well and incubate for 1 hour. Wash as before with 1X Wash buffer.

Add 100 µL of warm Substrate Solution and incubate for 2-20 minutes.

Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Read absorbance immediately on a microplate reader using 450 nm.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at +4°C immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage condition
Protein Binding Strip Well Plate	96 well	+4°C
Anti-MG Antibody (1000X)	10 µL	-20°C
Secondary Antibody, HRP Conjugate (1000X)	20 µL	+4°C
Assay Diluent	50 mL	+4°C
10X Wash Buffer	100 mL	+4°C
Substrate Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
MG-BSA Standard	75 µL	-20°C
MG Conjugate	20 µL	-20°C
100X Conjugate Diluent	300 µL	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at O.D. 450 nm (620 nm as optional reference wave length)

6. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.
- Any components not listed here are ready to use as supplied.

6.1 MG Conjugate Coated Plate

Δ Note: The MG Conjugate coated wells are not stable and should be used within 24 hours after coating. Only coat the number of wells to be used immediately.

6.1.1 Immediately before use, prepare 1X Conjugate Diluent by diluting the 100X Conjugate Diluent in 1X PBS. Example: Add 50 µL to 4.95 mL of 1X PBS.

6.1.2 Immediately before use, prepare 500 ng/mL of MG Conjugate by diluting the 1.0 mg/mL MG Conjugate in 1X Conjugate Diluent in two step dilutions. Example: Add 5 µL of 1.0 mg/Ml MG Conjugate to 995 µL of 1X PBS, vortex thoroughly, and transfer 500 µL to another tube containing 4.5 mL of 1X Conjugate Diluent.

6.1.3 Add 100 µL of the 500 ng/mL MG Conjugate to each well and incubate overnight at 4°C.

6.1.4 Remove the MG Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid.

6.1.5 Add 200 µL of Assay Diluent to each well and block for 1 hour at room temperature. Transfer the plate to 4°C and remove the Assay Diluent immediately before use.

6.2 1X Wash Buffer:

6.2.1 Dilute the 10X Wash Buffer Concentrate to 1X with deionized water.

6.2.2 Stir to homogeneity.

6.3 Anti-MG Antibody and Secondary Antibody

6.3.1 Immediately before use dilute the Anti-MG antibody 1:1000 and Secondary Antibody 1:1000 with Assay Diluent.

Δ Note: Do not store diluted solutions.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Prepare a dilution series of MG-BSA standards in the concentration range of 0 to 25 µg/mL by diluting the 1 mg/mL MG-BSA standard in Assay Diluent. Example: Add 10 µL to 390 µL of Assay Diluent.

7.2 Further prepare a series of MG-BSA standards according to table below:

Standard #	1 mg/mL MG-BSA Standard (µL)	Assay Diluent (µL)	MG-BSA (µg/mL)
1	10	390	25
2	200 of standard #1	200	12.5
3	200 of standard #2	200	6.25
4	200 of standard #3	200	3.13
5	200 of standard #4	200	1.56
6	200 of standard #5	200	0.78
7	200 of standard #6	200	0.39
8	200 of standard #7	200	0.20
9	0	200	0

8. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.

9. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- If testing mouse or rat plasma or serum, the IgG must be completely removed from each sample prior to testing, such as with Protein A or G beads. Additionally, a control well without primary antibody should be run for each sample to determine background signal.

9.1 Add 50 µL of unknown sample or MG-BSA standard to the wells of the MG Conjugate coated plate. If needed, unknown samples may be diluted in 1X PBS containing 0.1% BSA before adding. Incubate at room temperature for 10 minutes on an orbital shaker.

9.2 Add 50 µL of the diluted anti-MG antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.

9.3 Wash 3 times with 250 µL of 1X Wash Buffer with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.

9.4 Add 100 µL of the diluted Secondary Antibody-HRP Conjugate to all wells and incubate for 1 hour at room temperature on an orbital shaker. Wash the strip wells 5 times according to step 9.3 above.

9.5 Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well. Incubate at room temperature for 2-20 minutes on an orbital shaker.

Δ Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9.6 Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).

9.7 Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

10. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

10.1 Average the duplicate reading for each standard, control and sample.

10.2 If significant, subtract the sample background control from sample readings.

10.3 Plot the values for each standard as a function of the final concentration of MG Adduct.

10.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

10.5 Apply the sample OD reading to the standard curve to get MG Adduct amount in the sample wells.

10.6 Concentration of MG Adduct in [B units / V units] in the test samples is calculated as:

$$MG\ Adduct\ concentration = \frac{B}{V} * D$$

Where:

B = amount of MG Adduct in the sample well calculated from standard curve in µL/mL

V = sample volume added in the sample wells in µL

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

11. Typical Data

Typical standard curve - data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

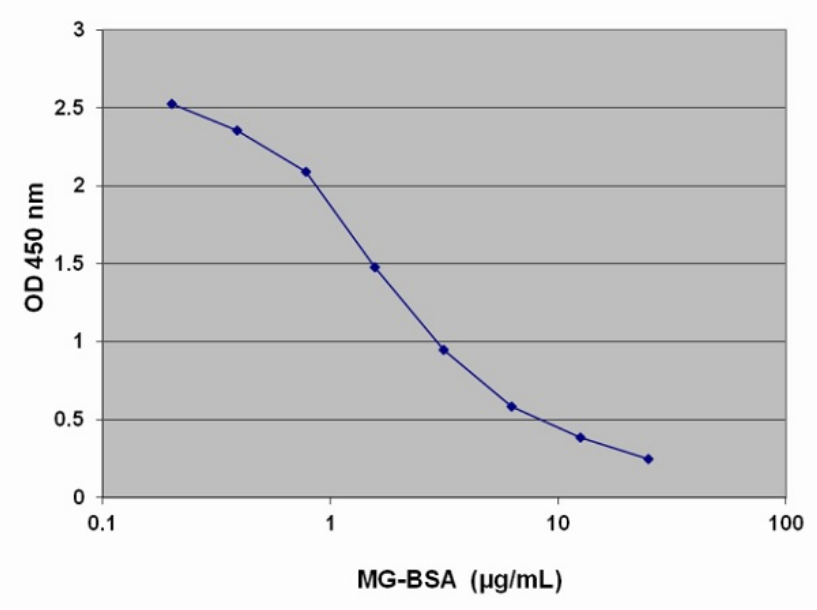

Figure 1. Typical Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

12. Species and Cross Reactivity

This kit is not species specific and can be used with samples from any species.

Cross reactivity of Methylglyoxal (MG) Competitive ELISA Kit

AGEs	Cross Reactivity (%)
MG-BSA	100
AGE-BSA*	2.3
CML-BSA	<0.001
CEL-BSA	<0.001
BSA	<0.001
Ovalbumin	<0.001

* AGE-BSA is prepared by incubating BSA with D-Glucose at 37°C for 6 weeks under sterile conditions.

Please contact our Technical Support team for more information.

13. Notes

Technical Support

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