Product Manual

Homocysteine ELISA Kit

Catalog Number

MET-5209 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Homocysteine is an amino acid intermediate formed during the production of the essential dietary amino acid methionine (Figure 1). Homocysteine is a homologue of cysteine, differing from cysteine only in that it contains an extra side chain methylene bridge. About 80% of homocysteine found in plasma is bound to protein. High levels of homocysteine in the blood have been associated with premature incidences of vascular disease, and homocysteine is likely to be a risk factor for heart disease. Homocysteine initially stimulates the production of nitric oxide in endothelial cells but ultimately reduces nitric oxide bioavailability and increases oxidative stress by blocking glutathione peroxidase activity as well as causing cellular oxidative degradation (increasing free radical generation). In addition, elevated homocysteine levels lead to increased platelet and leukocyte adhesion and activation, increased vasoconstriction, and increased proliferation of smooth muscle (a hallmark of atherosclerosis).

Cell Biolabs' Homocysteine ELISA Kit is a competitive enzyme immunoassay developed for the detection and quantitation of homocysteine in plasma, serum, lysates, or other biological fluid samples. The kit has a detection sensitivity limit of 10 ng/mL Homocysteine-Gelatin. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

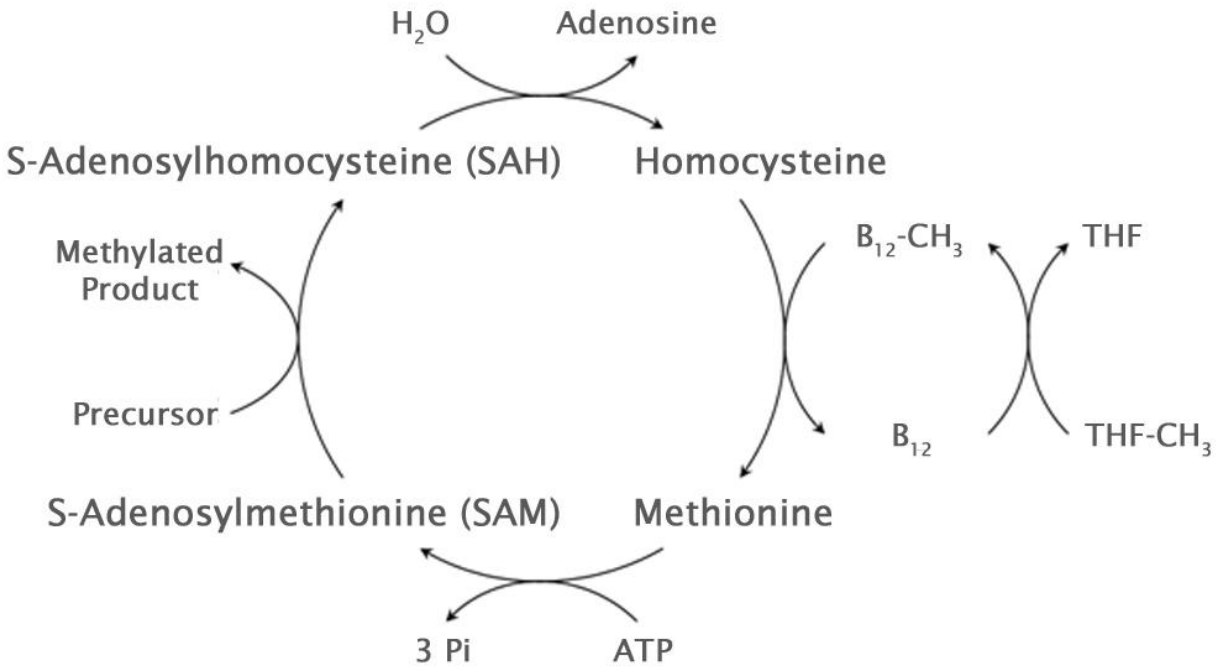

Figure 1. Metabolism of homocysteine.

Related Products

1. MET-5054: L-Amino Acid Assay Kit (Colorimetric)
2. MET-5151: S-Adenosylhomocysteine (SAH) ELISA Kit
3. MET-5152: S-Adenosylmethionine (SAM) ELISA Kit
4. MET-5158: Methionine Assay Kit
5. STA-800: OxiSelect™ Intracellular Nitric Oxide Assay Kit (Fluorometric)

Kit Components

Box 1 (shipped at room temperature)

1. 96 Well Protein Binding Plate (Part No. 231001): One strip well 96 well plate.
2. Anti-Homocysteine Antibody (500X) (Part No. 52093C): One 15 µL vial.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 231009): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Homocysteine Conjugate (100X) (Part No. 52091C): One 100 µL vial.
2. Homocysteine-Gelatin Standard (Part No. 52092C): One 80 µL vial of 1 mg/mL homocysteine conjugated to Gelatin in PBS.

Materials Not Supplied

1. Plasma, serum, or other biological fluids
2. Phosphate Buffered Saline (PBS)
3. PBS containing 0.1% Bovine Serum Albumin (BSA)
4. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, store Homocysteine Conjugate (100X) and Homocysteine-Gelatin Standard at -20ºC. Store the remainder of the kit at 4ºC.

Preparation of Reagents

- Homocysteine Conjugate Coated Plate: Determine the number of wells to be used, and dilute the Homocysteine Conjugate 1:100 into PBS. Add 100 µL of the diluted homocysteine conjugate to each well of the 96-well Protein Binding Plate. Incubate overnight at 4ºC. Remove the

homocysteine conjugate and wash wells 3 times with 200 µL of PBS. Blot on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hour at room temperature. Transfer the plate to 4ºC.

Note: The Homocysteine Conjugate Coated Plate is not stable long-term. Use the prepared plate within 24 hours after coating.

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-Homocysteine Antibody and Secondary Antibody, HRP Conjugate: Immediately before use dilute the Anti-Homocysteine Antibody 1:500 and the Secondary Antibody, HRP Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of Homocysteine-Gelatin standards in the concentration range of 0 to 40 µg/mL in Assay Diluent (Table 1).

Standard Tubes	1 mg/mL Homocysteine-Gelatin Standard (µL)	Assay Diluent (µL)	Homocysteine-Gelatin (µg/mL)
1	16	384	40
2	100 of Tube #1	300	10
3	100 of Tube #2	300	2.5
4	100 of Tube #3	300	0.625
5	100 of Tube #4	300	0.156
6	100 of Tube #5	300	0.039
7	100 of Tube #6	300	0.010
8	0	300	0

Table 1. Preparation of Homocysteine-Gelatin Standards.

Preparation of Samples

- Plasma: Collect blood with heparin or EDTA and centrifuge for 10 minutes at 1000 g at 4ºC. Remove the plasma and assay immediately or store samples at -80ºC for up to three months. Normal plasma samples should be diluted 2- to 10-fold with PBS containing 0.1% BSA immediately before running the ELISA.
- Serum: Harvest serum and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Normal serum samples should be diluted 2- to 10-fold with PBS containing 0.1% BSA immediately before running the ELISA.
- Tissue homogenate: Weigh and homogenize the tissue on ice in 5-10 mL cold PBS per gram of tissue. Centrifuge at 10,000 x g for 15 minutes at 4°C. Remove the supernatant and store on ice. Store any unused supernatant at -80°C for up to three months.
- Cell lysate: Collect cells by centrifuging at 2000 x g for 10 minutes at 4°C. Sonicate or homogenize the cell pellet on ice in 1-2 mL cold PBS. Centrifuge at 10,000 x g for 15 minutes at

4°C. Remove the supernatant and store on ice. Aliquot and store the supernatant for use in the assay. Store any unused supernatant at -80°C for up to three months.

- Other biological fluids: Centrifuge samples for 10 minutes at 1000 g at 4ºC and recover supernatant. Assay immediately or store samples at -80ºC for up to three months.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Each unknown sample and Homocysteine-Gelatin standard should be assayed in duplicate.
3. Immediately before use, remove the Assay Diluent from the plate and add 50 µL of unknown sample or standard to the Homocysteine Conjugate Coated Plate. Incubate at room temperature for 10 minutes on an orbital shaker.
4. Add 50 µL of diluted Anti-Homocysteine Antibody (see Preparation of Reagents section) to each well. Incubate at room temperature for 1 hour on an orbital shaker.
5. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After each wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
6. Add 100 µL of the diluted Secondary Antibody, HRP Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker. During this incubation, warm Substrate Solution to room temperature.
7. Wash the strip wells 3 times according to step 5 above. Proceed immediately to the next step.
8. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometric microplate reader using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Homocysteine Competitive ELISA Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

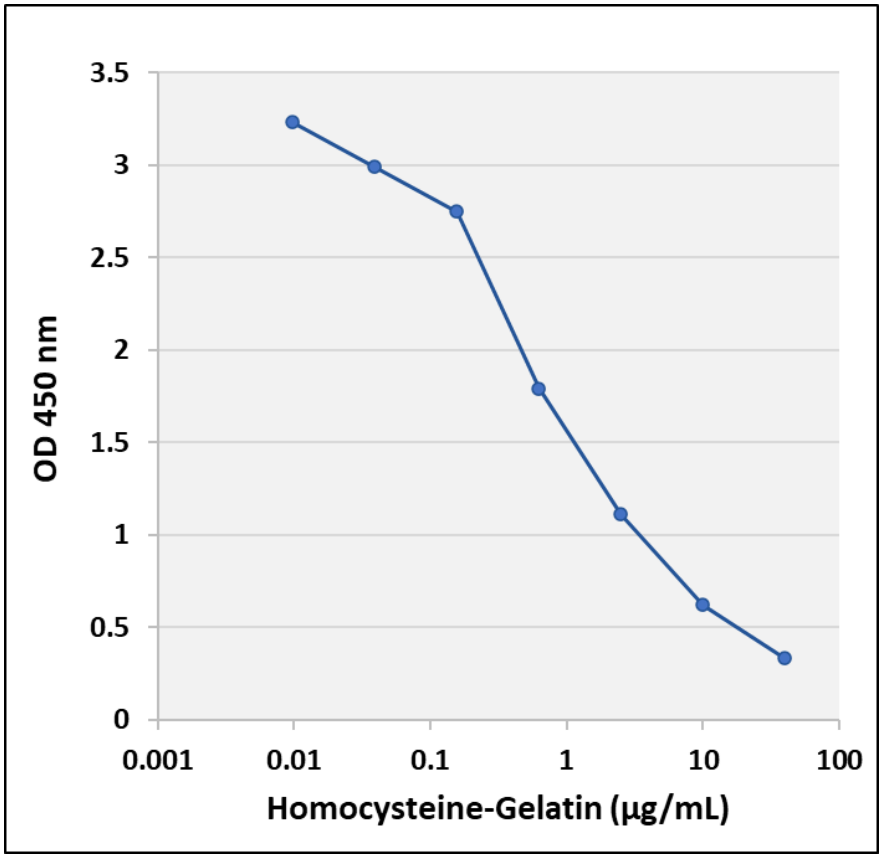

Figure 2: Homocysteine-Gelatin Standard Curve.

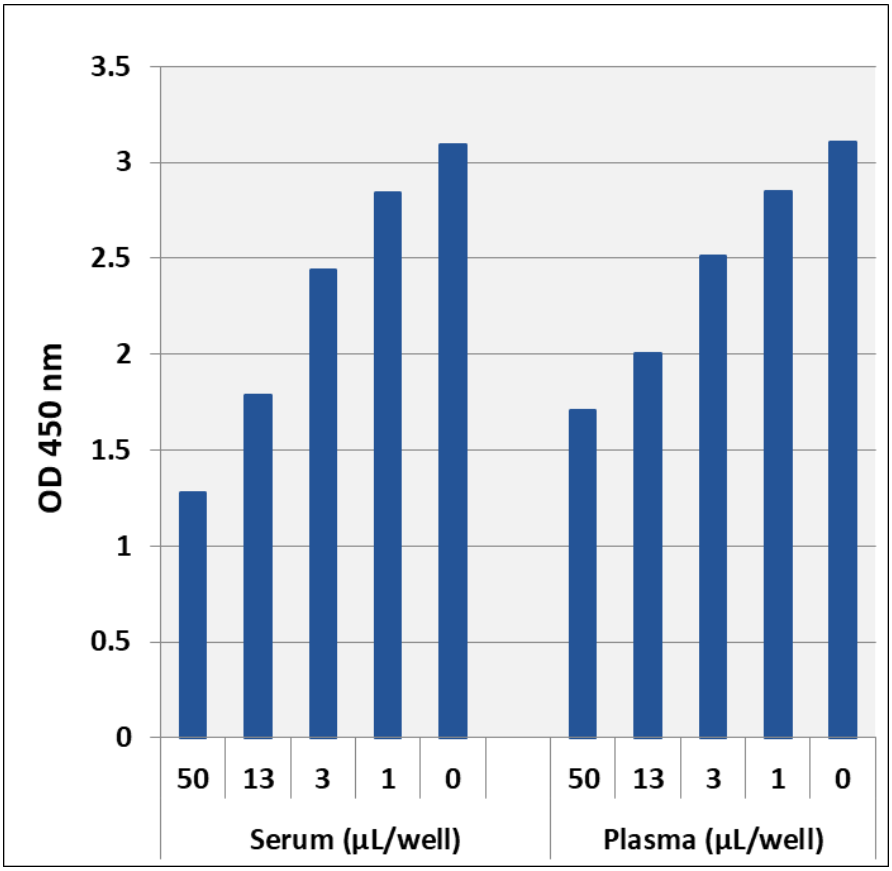

Figure 3: Homocysteine Detection in Human Serum and Plasma.

References

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3. Loscalzo J (1996) *J. Clin Invest*. **98**:5-7.
4. Upchurch GR, Jr., Welch GN Fabian AJ, Freedman JE, Johnson JL, Keaney JF Jr, and Loscalzo J. (1997) *J. Biol Chem* **272**:17012-17017.
5. Tyagi SC (1998) *Am. J. Physiol*. **274** (2 Pt 1): C396-405.

Warranty

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