Product Manual

Lipoic Acid ELISA Kit

Catalog Number

MET-5207 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Lipoic acid, also known as thioctic acid, is an organosulfur compound found in both free and bound forms. Lipoic acid is an enzyme cofactor when covalently bound to proteins through a lysine residue, where it acts as an intermediate step in enzymatic processes. The cofactor is essential for the function of several key enzymes involved in oxidative metabolism including pyruvate dehydrogenase (PDH), 2-oxoglutarate dehydrogenase, the branched-chain 2-oxoacid dehydrogenases, and the glycine cleavage system. Lipoic acid is also a known antioxidant.

Lipoic acid can be obtained either through normal diet or via lipoic acid supplements; it can also be synthesized in the body from octanoic acid. Mutations in the lipoic acid synthetase gene can result in lipoic acid deficiency. Lipoic acid synthetase deficiency in humans can result in hyperglycemia, lactic acidosis and seizures.

Cell Biolabs' Lipoic Acid ELISA Kit is a competitive enzyme immunoassay developed for the detection and quantitation of total bound lipoic acid in biological samples. This assay is not recommended for detection of free lipoic acid at very high concentrations in samples. The kit has a detection sensitivity limit of 4 nM bound lipoic acid. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. MET-5151-C: S-Adenosylmethionine (SAM) and S-Adenosylhomocysteine ELISA Combo Kit
2. MET-5090: Adenosine Assay Kit (Fluorometric)
3. MET-5014: NAD+/NADH Assay Kit (Colorimetric)
4. MET-5163: ATP 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. Secondary Antibody, HRP Conjugate (1000X) (Part No. 231009): One 20 µL vial
3. Assay Diluent (Part No. 310804): One 50 mL bottle
4. 10X Wash Buffer (Part No. 310806): One 100 mL bottle
5. Substrate Solution (Part No. 310807): One 12 mL amber bottle
6. Stop Solution (Part. No. 310808): One 12 mL bottle
7. 10X Coating Buffer (Part No. 52074C): One 2 mL vial

Box 2 (shipped on blue ice)

1. Lipoic Acid Conjugate (100X) (Part No. 52071D): One 100 µL vial
2. Lipoic Acid-PDH Standard (Part No. 52072D): One 100 µL vial of 90 µM of bound Lipoic Acid, provided in 30 µM of PDH E2, and each PDH molecule contains 3 lipoic acids
3. Anti-Lipoic Acid Antibody (1000X) (Part No. 52073D): One 10 µL vial

Materials Not Supplied

1. Phosphate Buffered Saline (PBS)
2. PBS containing 0.1% Bovine Serum Albumin (BSA)

Storage

Upon receipt, store Lipoic Acid Conjugate, Lipoic Acid-PDH Standard, Anti-Lipoic Acid Antibody at -20 ºC. Store the remainder of the kit at 4ºC.

Preparation of Reagents

- 1X Coating Buffer: Immediately before use, dilute the 10X Coating Buffer to 1X with deionized water. Stir to homogeneity.
- Lipoic Acid Conjugate Coated Plate: Dilute the Lipoic Acid Conjugate 1:100 into 1X Coating Buffer. Add 100 µL of 1X Lipoic Acid Conjugate to each well of the 96-well Protein Binding Plate. Incubate overnight at 4ºC. Remove the solution and wash wells 3 times with 200 µL of PBS. Blot plate 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 and remove the Assay Diluent **immediately before use**.

  Note: The Lipoic Acid 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.

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-Lipoic Acid Antibody and Secondary Antibody HRP Conjugate: Immediately before use dilute the Anti-Lipoic Acid Antibody 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 Lipoic Acid-PDH Standards in the concentration range of 0 to 3 µM in Assay Diluent (Table 1)

Standard Tubes	90 µM Lipoic Acid-PDH Standard (µL)	Assay Diluent or Desired Buffer (µL)	Lipoic Acid (nM)
1	10	290	3000
2	100 of Tube #1	200	1000
3	100 of Tube #2	200	333
4	100 of Tube #3	200	111
5	100 of Tube #4	200	37
6	100 of Tube #5	200	12
7	100 of Tube #6	200	4
8	0	200	0

Table 1. Preparation of Lipoic Acid-PDH Standards.

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- 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. Each unknown sample (see Preparation of Samples section), Lipoic Acid-PDH Standard, and blank should be assayed in duplicate.
2. Add 50 µL of unknown sample or standard to the Lipoic Acid Conjugated Coated Plate. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 µL of diluted Anti-Lipoic Acid Antibody (see Preparation of Reagents section) to each well. Incubate at room temperature for 2 hours on an orbital shaker.
4. 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.
5. 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.
6. Wash the strip wells 3 times according to step 5 above. Proceed immediately to the next step.
7. 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.

8. 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).
9. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

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

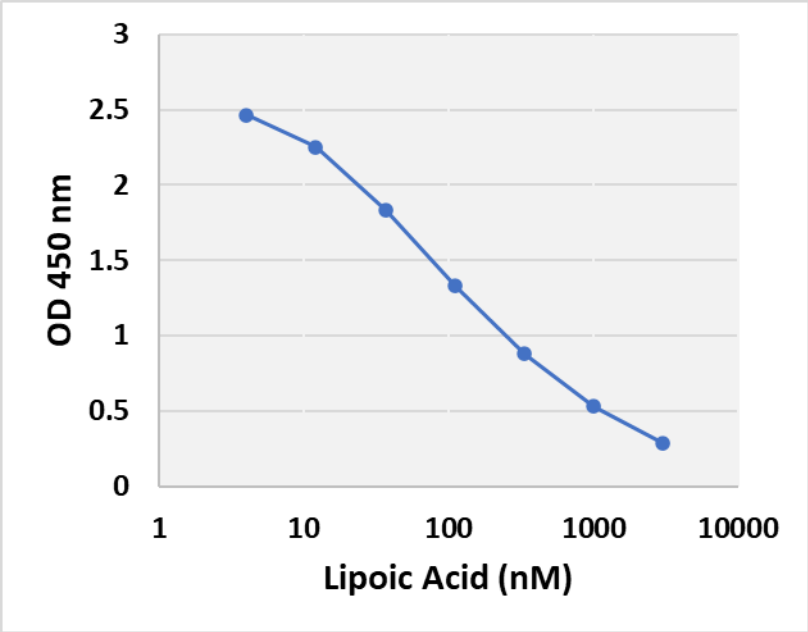

Figure 1: Lipoic Acid-PDH Standard Curve.

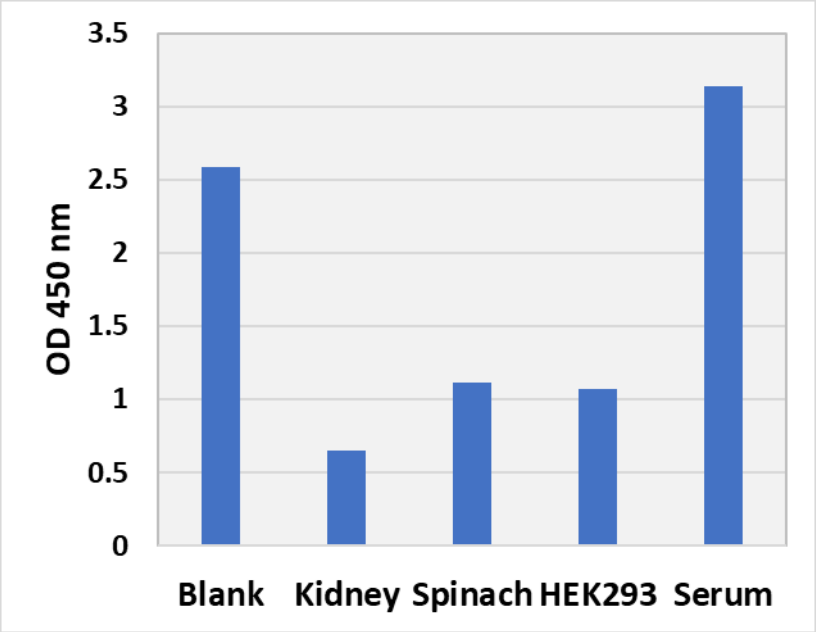

Figure 2: Lipoic Acid Detection. Lipoic Acid ELISA was performed with 1 mg/ml total protein from pig kidney, spinach leaves, HEK293 cells, and human sera.

References

1. Lodge JK and Packer L (1999) *Antioxidant Food Supplements in Human Health.* 121-134.
2. Mayr JA, Zimmerman FA, Fauth C, Bergheim, C, Meierhofer, D, Radmayr, D, Zschocke J, Koch J, and Wolfgang S (2011) *Am J Hum Genet.* **89**: 792-797.
3. Capece U, Moffa S, Improta I, Di Giuseppe G, Nista EC, Cefalo CMA, Cinti F, Pontecorvi A, Gasbarrini A, Giaccari A, and Mezza T (2023) *Nutrients* **15**: 18.
4. Mailloux RJ. (2024) *Redox Biology* **72**: 1-15.

Warranty

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