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Carnosine ELISA Kit

rev 01/20

(Catalog # E4766-100; 96 assays, Store kit at -20°C)

I. Introduction:

Carnosine is an imidazole dipeptide composed of the amino acids beta-alanine and histidine. Humans cannot produce carnosine and it must be obtained from meats such as beef, pork and chicken. Unlike simple amino acids, carnosine is not incorporated into proteins and is stored at relatively high concentrations (e.g. > 1 mg/ml) in brain, heart and muscle tissues. In humans, carnosine concentrations in plasma are very low during fasting but can jump to high levels after meat consumption. Interestingly, carnosine has several beneficial effects or therapeutic potential, like carnosine has a pKa of 6.8 which is ideal to buffer pH and delay fatigue in muscles. In addition, carnosine is an antioxidant, which can greatly scavenge reactive oxygen species (ROS) or alpha-beta unsaturated aldehydes formed during oxidative stresses or inflammations. Besides, carnosine has anti-glycating properties and it can reduce advanced glycation end-products in many degenerative diseases such as aging, atherosclerosis, chronic renal failure and alzheimer's disease. Carnosine is also important in diabetes as it can bind to excess sugars and lessen glucose-induced damage. The traditional techniques/instruments such as HPLC or GC-MS for detecting carnosine are expensive, laborious, and time-consuming. On the other hand, immunoassay techniques, such as ELISA, are commonly preferred as simple, reliable and rapid methods. **BioVision's Carnosine ELISA Kit** is a competitive ELISA that can detect carnosine in different biological samples such as serum and muscle. It can detect carnosine (0.25 – 16 µg/ml) within 120 minutes.

II. Applications:

In vitro quantitative determination of carnosine
Detection Range: 0.25- 16 µg/ml
Sensitivity: 0.12 µg/ml

III. Sample Type:

Serum and different tissues such as brain, heart and muscle etc.

IV. Kit Contents:

Components	E4766-100	Cap Code	Part Number
ELISA Microplate	8 X 12 Strips	--	E4766-100-1
Carnosine Standard	2 vials	Yellow	E4766-100-2
HRP Conjugate Stock	25 µl	Blue	E4766-100-3
Antibody	7 ml	NM/Red	E4766-100-4
TMB substrate	10 ml	Amber	E4766-100-5
Stop Solution	10 ml	NM/Blue	E4766-100-6
Wash Buffer (10X)	50 ml	NM	E4766-100-7
Sample Diluent	40 ml	NM/Red	E4766-100-8
Conjugate Buffer	7.5 ml	NM/Green	E4766-100-9
Plate Sealers	4	--	E4766-100-10

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm and 650 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards and sample dilutions
- PBS buffer

VI. Storage and Handling:

The entire kit may be stored at -20°C for up to 12 months from the date of shipment. Opened kit is stable for 2 months at 4°C.

VII. Reagent and Standard Preparation:

Bring all reagents to room temperature (RT) before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Antibody, TMB Substrate, Stop Solution, Sample Diluent and Conjugate Buffer:** Ready to be used. After use, store them at 4°C.
- **HRP Conjugate Stock:** Spin briefly before opening the tube. Pipet 5 µl of HRP Conjugate Stock into Conjugate Buffer (7.5 ml) bottle to prepare Conjugate working solution. Vortex the bottle for a minute. The conjugate working solution is stable at 4°C for 2 months.
- **Wash Buffer (10X)**: Bring bottle to RT. If crystals are present, warm up to RT and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X solution can be stored at 4°C for one month.
- **Carnosine Standard:** Reconstitute the Carnosine Standard by adding 0.9 ml of water to prepare 16 µg/ml Standard (S7). Allow solution to sit at RT for 10 minutes, then gently vortex to mix completely. Perform 2-fold serial dilutions from S7 (e.g. mix 0.5 ml Standard with 0.5 ml of water) to prepare Standards S6 to S1 sequentially. S0 is water only. The Standards are stable at -20°C for up to 3 weeks.

Standards	S0	S1	S2	S3	S4	S5	S6	S7
Concentrations (µg/ml)	0	0.25	0.5	1	2	4	8	16

VIII. Sample Preparation:

- **Serum**

1. Dilute the serum sample 20-fold using the Sample Diluent (e.g. mix 10 µl of serum with 190 µl of Sample Diluent.)
2. Use 50 µl per well for the assay.

- **Muscle**

1. Transfer 100 mg of tissue to an eppendorf tube containing 0.5 ml of cold PBS in an ice bucket.
2. Homogenize the tissue using a small pestle for 5 min.
3. Let the tube incubated in the ice bucket for 5 min.
4. Centrifuge the tube at 10,000 x *g* and 4°C for 10 min and collect the supernatant.
5. Dilute the supernatant 100-fold with Sample Diluent.
6. Use 50 µl per well for the assay.

IX. Carnosine ELISA Assay Protocol:

Notes: We recommended that all standards and samples are run in duplicate. A Standard curve must be run each time an assay is performed.

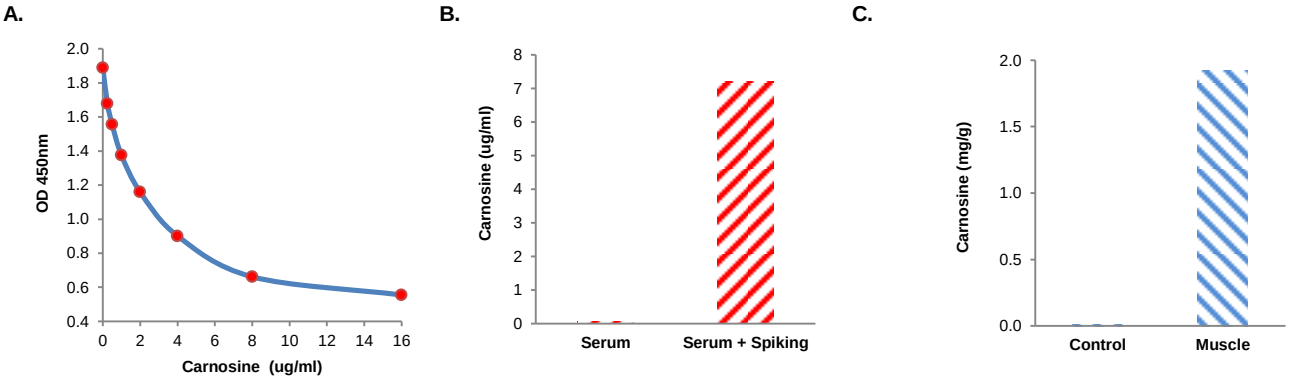
1. Prepare all reagents, standards and samples as sections VII and VIII specify respectively.
2. Add 50 µl of **Standards or Samples** per well. Then add 50 µl of **Conjugate working solution** and 50 µl of **Antibody** to the above wells.
3. Cover the microtiter plate with plate sealer and mix well. Incubate the plate at room temperature (25°C) for 75 min.
4. Aspirate all reagents and wash each well 5 times: add 250 µl of **1X Wash Buffer** and incubate for 30 seconds. Remove 1X Wash buffer completely before the next wash. (Complete removal of wash buffer is essential for accurate results.) Repeat wash step 4 more times.
5. Add 100 µl of **TMB Substrate** to each well. Tap or shake the plate to ensure complete mixing.
6. Check the OD at 650 nm for the well containing no carnosine (S0). When its reading is approximately 0.8 (usually between 5-30 min after adding the TMB Substrate), add 50 µl of **Stop Solution** and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm.

X. Calculation:

The mean values of relative absorbance are divided by the absorbance value of the zero-standard (A_0) and multiplied by 100%. The zero-standard is set to 100% and the relative absorbance of the standards and samples (A) are expressed as percentages.

Relative Absorbance (%) = A/A_0 X 100%
A: The average absorbance of the Standards or Samples
A_0 : The average absorbance of the zero Standard

The Standard Curve is done by plotting the relative absorbance of the Standards vs. Carnosine concentrations. The concentration of carnosine of each sample, which can be read from the calibration curve is multiplied by the corresponding dilution factor.



Figures. **A.** Carnosine Standard Curve (*This Standard Curve is for demonstration only. A Standard Curve must be run with each assay*). **B.** Spike recovery experiment: Human serum sample was assayed with and without carnosine spike (8 µg/ml) and showed 90% recovery. **C.** Carnosine concentration in muscle tissue was determined by using the assay kit.

XI. RELATED PRODUCTS:

Gentamicin (serum/urine) ELISA Kit (K4315)
Folic Acid ELISA Kit (E4523)
Caffeine Acid ELISA Kit (E4558)
His-Tag Protein ELISA Kit (E4550)
DYKDDDDK-Tag Protein ELISA Kit (E4700)
Ampicillin ELISA Kit (E4350)
Quinolone ELISA Kit (E4530)
Vancomycin ELISA Kit (E4605)
GST Tag ELISA Kit (E4690)
Bisphenol A ELISA Kit (E4722)

FOR RESEARCH USE ONLY! Not to be used on humans.