Product Manual

Choline Assay Kit (Colorimetric)

Catalog Number

MET-5043 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Choline is a water soluble amine that is an essential nutrient. Choline and its metabolites are important to cell signaling, cholinergic neurotransmission, and cell membrane structural integrity. The metabolite is categorized as a B-complex vitamin that is synthesized by the human body, but not in sufficient quantity for proper health. It is a key precursor to the phospholipids phosphatidylcholine and sphingomyelin, which are abundant in cell membranes. During the perinatal period, choline is transferred to infants via breast milk to assist in brain and growth development. The neurotransmitter acetylcholine, which is intricate to the central and peripheral nervous systems, is produced from choline.

Choline disorders can have a profound impact on neurological function. Choline deficiency has been implicated in atherosclerosis, liver disease, cancers, as well as neurological disorders. Neural tube and memory defects in infants have been linked with low choline levels in pregnant women. More research of choline is needed to elucidate its complex roles within the body and its relation to disease states.

Cell Biolabs' Choline Assay Kit is a simple colorimetric assay that measures the amount of choline present in plasma or serum, tissue homogenates, or cell suspensions in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 96 assays, including blanks, standards and samples. Sample choline concentrations are determined by comparison with a known choline standard. The kit's detection sensitivity limit is approximately 750 nM choline.

Assay Principle

Cell Biolabs' Choline Assay Kit measures choline present within serum, plasma, and other tissue samples. The assay is based on an enzyme driven reaction that will detect choline via choline oxidase activity. Choline is oxidized by choline oxidase to produce hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific colorimetric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples are compared to a known concentration of choline standard within the 96-well microtiter plate format. Samples and standards are incubated for 60 minutes and then read with a standard 96-well spectrophotometric microplate reader in the 540-570 nm range (Figure 1).

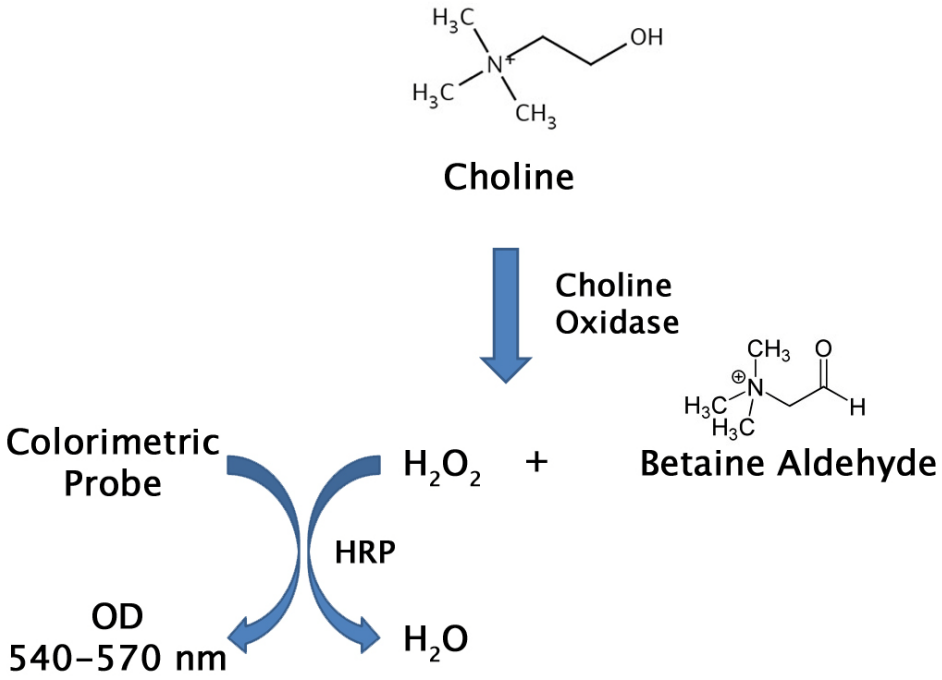

Figure 1. Choline Assay Principle.

Related Products

1. MET-5042: Choline Assay (Fluorometric)
2. STA-384: Total Cholesterol Assay Kit (Colorimetric)
3. STA-390: Total Cholesterol Assay Kit (Fluorometric)
4. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
5. STA-394: HDL Cholesterol Assay Kit
6. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
7. STA-600: Phosphatidylcholine Assay Kit
8. STA-601: Sphingomyelin Assay Kit
9. STA-602: Acetylcholine Assay Kit (Fluorometric)
10. STA-603: Acetylcholine Assay Kit (Colorimetric)

Kit Components

1. Choline Standard (Part No. 50421C): One 50 µL tube of 20 mM choline.
2. Assay Buffer (20X) (Part No. 50422A): One 25 mL bottle.
3. Colorimetric Probe (50X) (Part No. 260301): One 100 µL tube in DMSO.

4. HRP (Part No. 234402): One 100 µL tube of a 100 U/mL solution in glycerol.
5. Choline Oxidase (Part No. 260205): One 25 µL tube.

Materials Not Supplied

1. Distilled or deionized water
2. 1X PBS
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Spectrophotometric microplate reader capable of reading in the 540-570 nm absorbance range
7. Centrifugal filters for plasma or serum samples (e.g. Millipore Amicon® Ultra-0.5mL, Ultracel® membrane filters, or Thermo Pierce Concentrators PES membrane filters)
8. (optional) Superoxide dismutase

Storage

Upon receipt, store the Assay Buffer at 4ºC. Store the remaining kit components at -20ºC. The Colorimetric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

- 1X Assay Buffer: Warm the Assay Buffer (20X) to room temperature prior to using. Dilute the Assay Buffer (20X) with deionized water by diluting the 25 mL bottle of buffer with 475 mL deionized water for 500 mL total. Mix to homogeneity. Store the 1X Assay Buffer at 4ºC up to six months.
- Choline Reaction Reagent: Prepare a reaction reagent to test for choline by diluting the Choline Oxidase 1:200, HRP 1:500, Colorimetric Probe 1:50 in 1X Assay Buffer. (e.g. For 50 assays, combine 12.5 µL of Choline Oxidase, 5 µL of HRP, 50 µL Colorimetric Probe with 1X Assay Buffer to 2.5 mL total solution). Mix thoroughly and protect the solution from light. For best results, place the Choline Reaction Reagent on ice and use within 30 minutes of preparation. Do not store the Choline Reaction Reagent solution.

Preparation of Samples

Samples should be assayed immediately or stored at -80ºC prior to performing the assay. Optimal experimental conditions for samples must be determined by the investigator. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering compounds. Use clear supernatants in the assay. Run proper controls as necessary. Always run a standard curve with samples.

- Tissue lysates: Sonicate or homogenize tissue samples in PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. The supernatant may be assayed directly or diluted as necessary in 1X Assay Buffer.

- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS. Homogenize or sonicate the cells on ice. Centrifuge at 14,000 rpm for 5-10 minutes to remove debris. Cell lysates may be assayed directly or diluted as necessary in 1X Assay Buffer.
- Urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed directly or diluted as necessary in 1X Assay Buffer.
- Milk: Milk samples should be homogenous and cleared by mixing 600 µL milk with 100 µL 6 N HCl. Centrifuge at 14,000 rpm for 5-10 minutes. Transfer 300 µL of the supernatant into a clean tube and neutralize with NaOH. The neutralized supernatant is ready for assay.
- Serum: Collect blood without using an anticoagulant. Allow blood to clot for 30 minutes at room temperature. Centrifuge at 2000 x g and 4ºC for 10 minutes. Remove the serum layer and store on ice. Take care to avoid disturbing the white buffy layer. Aliquot samples for testing and store remaining solution at -80ºC. Prior to testing, it is recommended to deproteinize samples by filtering with a 3K-10K centrifugal filter (e.g. Millipore Amicon® Ultra-0.5mL, Ultracel® membrane filters, or Thermo Pierce Concentrators PES membrane filters). Perform serum dilutions in 1X Assay Buffer.
- Plasma: Collect blood with heparin or citrate and centrifuge at 1000 x g and 4ºC for 10 minutes. Remove the plasma layer and store on ice. Take care to avoid disturbing the white buffy layer. Aliquot samples for testing and store remaining solution at -80ºC. Prior to testing, it is recommended to deproteinize samples by filtering samples with a 3K-10K centrifugal filter (e.g. Millipore Amicon® Ultra-0.5mL, Ultracel® membrane filters, or Thermo Pierce Concentrators PES membrane filters). Perform plasma dilutions in 1X Assay Buffer.

  Notes:

  1. *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL.*
  2. *Avoid samples containing DTT or β-mercaptoethanol since the probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Choline Standard Curve

1. Prepare fresh choline standards by first diluting a portion of the 20 mM Choline Standard stock solution 1:100 in 1X Assay Buffer. (e.g. Add 10 µL of Choline Standard stock in 990 µL 1X Assay Buffer). Vortex thoroughly. This provides a 200 µM concentration. Use this 200 µM solution to prepare a series of the remaining choline standards according to Table 1 below.

Tubes	20 mM Choline Standard (µL)	1X Assay Buffer (µL)	Resulting Choline Concentration (µM)
1	10	990	200
2	250 of Tube #1	250	100
3	250 of Tube #2	250	50
4	250 of Tube #3	250	25
5	250 of Tube #4	250	12.5
6	250 of Tube #5	250	6.25
7	250 of Tube #6	250	3.13
8	250 of Tube #7	250	1.56
9	250 of Tube #8	250	0.78
10	0	500	0

Table 1. Preparation of Choline Standards.

Note: Do not store diluted choline standard solutions.

Choline Assay Protocol

Each choline standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 50 µL of the diluted choline standards or samples to the 96-well microtiter plate.
2. Add 50 µL of the prepared Choline Reaction Reagent to each standard and sample wells. Mix all well contents thoroughly.
3. Cover the plate wells to protect the reaction from light. Incubate the plate on an orbital rotator for 60 minutes at room temperature.
4. Read the plate with a spectrophotometric microplate reader in the 540-570 nm range.
5. Calculate the concentration of choline within samples by comparing the sample absorbance to the choline standard curve.

Example of Results

The following figures demonstrate typical Choline Assay results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

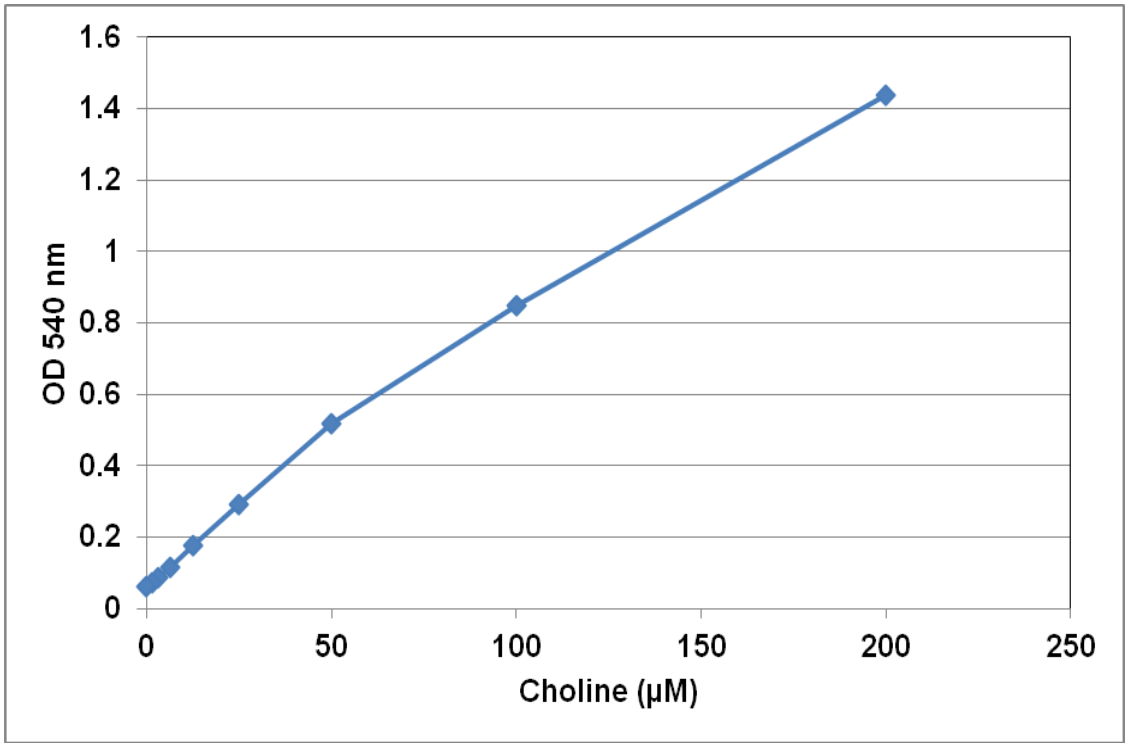

Figure 2: Choline Standard Curve.

Calculation of Results

1. Calculate the average absorbance values for every standard, control, and sample. Subtract the average zero standard value from itself and all standard and sample values. This is the corrected absorbance.
2. Plot the corrected absorbance for the standards against the final concentration of the choline standards from Table 1 to determine the best curve. See Figure 2 for an example standard curve.
3. Determine the choline concentration of the samples with the equation obtained from the linear regression analysis of the standard curve. Substitute the corrected absorbance values for each sample. Remember to account for dilution factors.

$$\text{Choline (µM)} = \left[\frac{\text{sample corrected absorbance}}{\text{slope}} \right] \times \text{sample dilution}$$

References

1. Holm, P.I., et al. (2003) *Clin. Chem.* **49**: 286-294.
2. Larsen, Torben, et al. (2010) *J. Dairy Res.* **77**: 438-444.
3. Ohta-Fukuyama, M., et al. (1980) *J. Biochem.* **88**: 197-203.
4. Woollard, D., et al. (2000) *J. AOAC Int.* **83**: 131-138.

Warranty

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