Choline Assay Kit (Colorimetric)

Choline Assay Kit (Colorimetric) is a detection kit for the quantification of Choline in serum, plasma, milk, urine, cell lysate and tissue lysate.

Catalog number: ARG82230

Package: 96 assays

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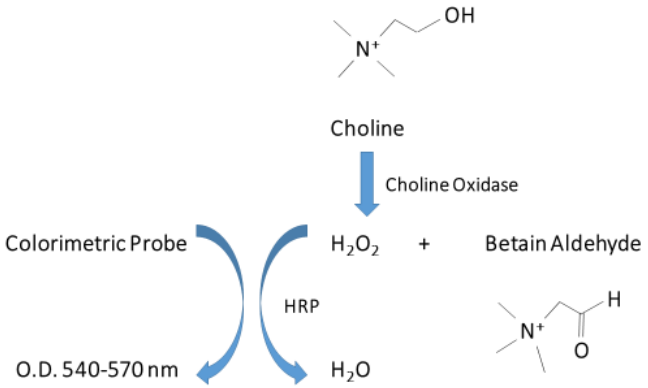
INTRODUCTION

Choline is an essential nutrient for humans and many other animals. Choline occurs as a cation that forms various salts (X^- in the depicted formula is an undefined counteranion). To maintain health, it must be obtained from the diet as choline or as choline phospholipids, like phosphatidylcholine. Humans and most animals make choline de novo, but production is insufficient in humans and most species. Choline is often not classified as a vitamin, but as a nutrient with an amino acid–like metabolism. In most animals, choline phospholipids are necessary components in cell membranes, in the membranes of cell organelles, and in very low-density lipoproteins. Choline is required to produce acetylcholine, a neurotransmitter and S-adenosylmethionine, a universal methyl donor involved in the synthesis of homocysteine.

Symptomatic choline deficiency (rare in humans) causes nonalcoholic fatty liver disease and muscle damage. Excessive consumption of choline (greater than 7.5 g/day) can cause low blood pressure, sweating, diarrhea and fish-like body odor due to trimethylamine, which forms in its metabolism. Rich dietary sources of choline and choline phospholipids include organ meats and egg yolks, dairy products and vegetables. [Provide by Wikipedia: Choline]

PRINCIPLE OF THE ASSAY

This Choline Assay Kit (Colorimetric) employs a simple colorimetric assay to measure the amount of choline in plasma, serum, tissue homogenates or cell suspensions. 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 and standards are incubated and then read with a microplate reader in the 540-570 nm range. The concentration of choline in the samples is then determined by comparing the 540-570 nm absorbance of samples to the standard curve.



MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Choline Standards (20 mM)	50 µL	-20°C
50X Colorimetric Probe	100 µL	-20°C (Protect from light)
Choline Oxidase	25 µL	-20°C
20X Assay Buffer	25 mL	4°C
HRP (100 U/mL)	100 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate Reader capable of reading in O.D. 540-570 nm range
- Standard 96-well microplate
- Superoxide dismutase (optional)
- Centrifugal filters for plasma or serum samples
- 1X PBS
- Deionized or Distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, store the Assay Buffer at 4°C. Store the remaining kit components at -20°C. The Colorimetric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze / thaw cycles.
- 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.
- Avoid samples containing DTT or β-mercaptoethanol since the probe is not stable in the presence of thiols (above 10 µM).
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

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. Run proper controls as necessary. Always run a standard curve with samples.

Tissue Lysates: Sonicate or homogenize tissue sample in cold PBS or 1X Assay Buffer and centrifuge at 10,000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay buffer.

Cell Lysates: Re-suspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge at 10,000 x g for 10 minutes at 4°C to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in 1X Assay Buffer.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes. The supernatant can be assayed directly or diluted as necessary in 1X Assay Buffer.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. The supernatant can be assayed directly or diluted as necessary in 1X Assay Buffer.

Urine: To remove insoluble particles, centrifuge at 2500 x g for 10 minutes. The supernatant can 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 10,000 x g for 10 minutes. Transfer 300 µL of the supernatant into a clean tube and neutralize with NaOH. The neutralized

supernatant is ready for assay.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum / plasma sample.
3. Samples should be assayed immediately or stored at -80°C prior to performing the assay. Optimal experimental conditions for samples must be determined by the investigator.
4. 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.
5. Avoid samples containing DTT or β-mercaptoethanol since the probe is not stable in the presence of thiols (above 10 µM).

REAGENT PREPARATION

- **1X Assay buffer:** Dilute the 20X Assay Buffer into deionized water to yield 1X Assay Buffer. (E.g., add 25 mL of 20X Assay Buffer into 475 mL of deionized water to a final volume of 500 mL) Mix to homogeneity. Store the 1X Assay Buffer at 4°C 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, Add 12.5 µL of Choline Oxidase, 5 µL of HRP and 50 µL Colorimetric Probe with 1X Assay Buffer to 2.5 mL total volume). 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.
- **Standards:** Prepare fresh Choline Standards by diluting in 1X Assay Buffer according to the Table below.

Standard tubes	Final Choline conc. (µM)	Volume of 1X Assay Buffer (µL)	Volume of Choline Standard (µL)
S1	200	990	10 of 20 mM Choline Standard
S2	100	250	250 of S1
S3	50	250	250 of S2
S4	25	250	250 of S3
S5	12.5	250	250 of S4
S6	6.25	250	250 of S5
S7	3.13	250	250 of S6
S8	1.56	250	250 of S7
S9	0.78	250	250 of S8
S0	0	250	0

ASSAY PROCEDURE

Prepare and mix all reagents thoroughly before use. Each 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 **samples** or serial **diluted Choline Standards** into 96-well microplate.
2. Add **50 µL** of **prepared Choline Reaction Reagent** to each well. Mix all contents thoroughly.
3. Cover the plate wells to protect the reaction from light. Incubate for **60 minutes** at **room temperature** on a microplate shaker.
4. Read the plate in the **540-570 nm** range with a microplate reader.
5. Calculate the concentration of choline within samples by comparing the sample absorbance to the choline standard curve.

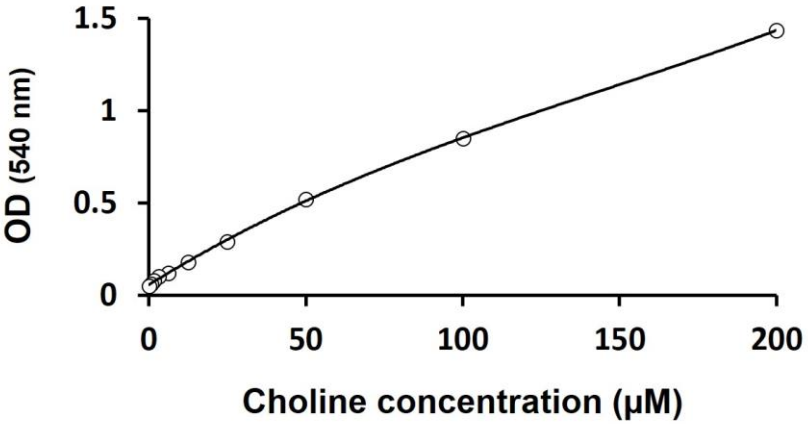
CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of standards and samples. Subtract the average zero standard value from itself and all standard and sample values. This is the corrected absorbance.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
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.

Choline (µM) = [sample corrected absorbance / slope] x sample dilution factor

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Choline Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

750 nM

Intra-assay and Inter-assay precision

The CV value of intra-assay and inter-assay was ≤ 10 %