

Choline/Acetylcholine Quantification Kit

(Catalog #K615-100; 100 assays; Store kit at -20°C)

I. Introduction:

Choline and acetylcholine play important roles in many biological processes. BioVision's Choline/Acetylcholine Quantification Kit provides a simple and sensitive means for quantifying Choline and Acetylcholine by either a colorimetric or fluorometric method. In the assay free choline is oxidized to betaine, via the intermediate betaine aldehyde. The reaction generates products which react with the Choline Probe to generate color ($\lambda=570$ nm), and fluorescence (Ex/Em 535/587 nm). Acetylcholine can be converted to choline by adding acetylcholinesterase to the reaction. The kit can detect choline and acetylcholine (total choline – free choline) in various biological samples such as in blood, cells, culture media, fermentation media, etc. There is no need for pretreatment or purification of samples. The kit can detect 10 pmol–5 nmol of choline or acetylcholine.

II. Kit Contents:

Components	100 Assays	Cap Code	Part Number
Choline Assay Buffer	25 ml	WM	K615-100-1
Choline Probe	1 Vial	Red	K615-100-2
DMSO (anhydrous)	400 μ l	Brown	K615-100-3
Choline Enzyme Mix	1 Vial	Green	K615-100-4
Acetylcholinesterase	1 Vial	Blue	K615-100-5
Choline Standard (5 μ mol)	1 Vial	Yellow	K615-100-6

III. Reagent Preparation and Storage Conditions:

Choline Probe: Dissolve in 220 μ l of anhydrous DMSO (provided). Mix well. Store at -20°C, protect from light and moisture. Use within two months.

Choline Enzyme Mix, Acetylcholinesterase: Dissolve in 220 μ l Choline Assay Buffer. Pipet up and down several times to dissolve. Store at -20°C. Use within two months.

Choline Standard: Dissolve in 100 μ l of Choline Assay Buffer to generate 50 nmol/ μ l of choline standard solution. Use within two month.

IV. Assay Protocol:

- Standard Curve Preparations:** For the colorimetric assay, dilute the Choline Standard to 0.5 nmol/ μ l by diluting 10 μ l of the Choline Standard into 990 μ l of Choline Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 μ l of the diluted standard choline into each well individually. Adjust volume to 50 μ l/well with Choline Assay Buffer to generate 0, 1, 2, 3, 4, 5 nmol/well of the Choline Standard.

For the fluorometric assay, dilute the Choline Standard to 50 pmol/ μ l. Then follow the same procedure as with the colorimetric assay, add 0, 2, 4, 6, 8, 10 μ l into each well individually. Adjust volume to 50 μ l/well with Choline Assay Buffer to generate 0, 100, 200, 300, 400, 500 pmol/well of the Choline Standard. If a more sensitive assay is desired, further dilute the standard 10 fold more, then follow the same procedure to make the standard curve at 0, 10, 20, 30, 40, 50 pmol/well. The fluorometric assay is 10 to 100 fold more sensitive than the colorimetric assay.

- Sample Preparation:** Prepare test samples in 50 μ l/well with Choline Assay Buffer in a 96-well plate. 1-10 μ l/assay of human serum can be tested (human serum contains

- ~10 μ M choline). Tissue or cells can be lysed in Choline Assay Buffer on ice for 10 min or by homogenization, then centrifuge to remove debris. The lysate can be tested directly.

Notes: We suggest using several dilutions of your sample to ensure the readings are within the standard curve range. Free choline in serum is known to increase upon storage due to breakdown of lipids.

- Reaction Mix Preparation:** Mix enough reagents for the number of assays performed. For each well, prepare a total 50 μ l Reaction Mix containing the following components:

44 μ l Choline Assay Buffer
 2 μ l Choline Probe
 2 μ l Acetylcholinesterase*
 2 μ l Enzyme Mix

*Note: Omit the acetylcholinesterase if you want to detect free choline only. With addition of Acetylcholinesterase, the assay detects total choline (free choline + acetylcholine).

- Add 50 μ l of the Reaction Mix to each well containing the Choline Standards or test samples, mix well. Incubate at room temperature for 30 min, protect from light.
- Measure O.D. at 570 nm for the colorimetric assay or measure fluorescence at Ex/Em = 535/590 nm in a micro-plate reader for fluorescence assay.
- Subtract background value (the 0 choline control) from all standard and sample readings. Plot standard curve nmol/well Vs. OD570nm or fluorescence readings. Then apply the sample readings to the standard curve to obtain choline amount in the sample wells. Calculate the choline concentrations of the test samples:

$$\text{Choline concentration} = \text{Cho}/\text{Sv} \text{ (nmol/ml or } \mu\text{M)}$$

Where: Cho is the choline amount (nmol) of your sample obtained from standard curve.
 Sv is the sample volume (ml) added to the sample well.

$$\text{Acetylcholine} = \text{total choline} - \text{free choline}$$

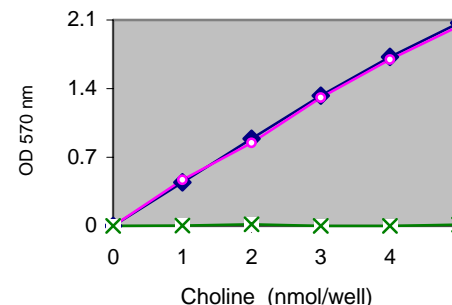


Fig. 1: Choline/Acetylcholine Assays were performed following the kit instructions. The square line is generated using choline as the substrate, whereas the open circle and the x lines were generated using acetylcholine as substrate in the presence and absence of acetylcholinesterase.

V. Related Products:

- Cholesterol Assay Kit
- Glutathione Assay Kit
- Glucose Assay Kit
- NADH/NAD, NADPH/NADP Assay Kit
- Amino Acid Quantification Kit
- Lactate Assay Kit
- Uric Acid Assay Kit
- Ascorbic Acid/Vitamin c Quantification Kit