APExBIO
An Apoptosis and Epigenetics Company

Product Information

Choline/Acetylcholine Quantification Colorimetric/Fluorometric Kit

I. Kit Contents:

Components	K2123-100 100 assays	Cap Color	Part Number
Choline Assay Buffer	25 ml	WM	K2123-C-1
Choline Probe	200 µl	Red	K2123-C-2
Choline Enzyme Mix	1 Vial	Green	K2123-C-3
Acetylcholinesterase	1 Vial	Blue	K2123-C-4
Choline Standard (5 µmol)	1 Vial	Yellow	K2123-C-5

II. Introduction:

Choline is a water-soluble nutrient. Acetylcholine (ACh) acts as a neurotransmitter in the autonomic nervous system (ANS). Choline and ACh play important roles in many biological processes.

The Choline/Acetylcholine Quantification Colorimetric/Fluorometric Kit provides a highly sensitive, fast and convenient way for detection of choline and acetylcholine in various biological samples such as blood, cells, fermentation media and culture media based on colorimetric and fluorometric method. In the assay, free choline is oxidized to betaine via the intermediate betaine aldehyde. The reaction generates products which react with Choline Probe to yield fluorescence (Ex/Em = 535/587 nm) and color (λ = 570 nm). Acetylcholine is converted to choline by acetylcholinesterase. The assay can detect choline and acetylcholine (total choline - free choline). The kit can detect 10 pmol~5 nmol of choline or acetylcholine without pretreatment or purification of samples.

III. Reagent Preparation and Storage Conditions:

Choline Probe: Ready to use as supplied. Warm up to room temperature before use to melt frozen DMSO. Store at -20°C, protect from light and moisture. Use within two months.

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

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

IV. Assay Protocol:

1. Standard Curve Preparations: For the colorimetric assay, dilute the Choline Standard to 0.5 nmol/µl by diluting 10 µl of the Choline Standard into 990 µl of Choline Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl of the diluted standard choline into each well individually. Adjust volume to 50 µl/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/µl. Then follow the same procedure as with the colorimetric assay, add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/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.

2. Sample Preparation: Prepare test samples in 50 µl/well with Choline Assay Buffer in a 96-well plate. 10 - 25 µl/assay of human serum can be tested (human serum contains ~ 10 µM 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.

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

Choline Assay Buffer	44 µl
Choline Probe	2 µl
Acetylcholinesterase	2 µl
Enzyme Mix	2 µl

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

4. Add 50 µl 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.

5. Measure OD at 570 nm for the colorimetric assay or measure fluorescence at Ex/Em = 535/590 nm in a micro-plate reader for fluorescence assay.

6. Subtract background value (the 0 choline control) from all standard and sample readings. Plot standard curve nmol/well Vs. OD_{570nm} 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:

Choline concentration = Cho/Sv (nmol/ml or µM)

Where: Cho is the sample choline amount determined from standard curve.

Sv is the sample volume (ml) added to the sample well.

Acetylcholine = total choline – free choline.

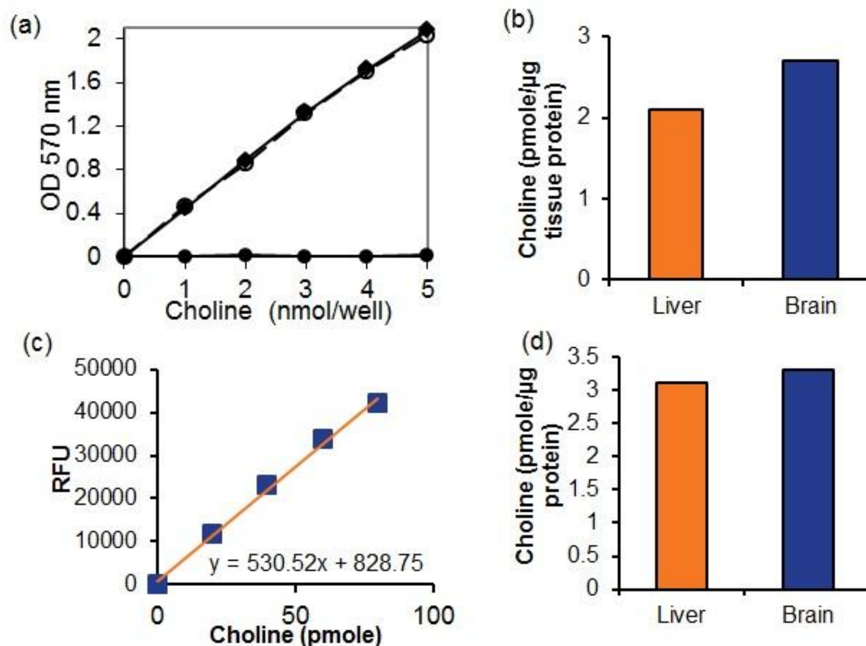

Figure: (a) Colorimetric Standard Curve. The diamonds are generated using choline as the substrate, whereas the open and closed circles were generated using acetylcholine as substrate in the presence and absence of acetylcholinesterase. (b) Choline estimation in rat liver (230 µg) and brain (150 µg) using colorimetric method. (c) Fluorometric Standard Curve (d) Choline estimation in rat liver (11.5 µg) and brain (7.5 µg) using fluorometric method. Choline levels in rat tissue is normalized to tissue protein concentration. Assays were performed following the kit protocol.

APExBIO
An Apoptosis and Epigenetics Company

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in d atasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

Tel: +1-832-696-8203; Fax: +1-832-641-3177
http://www.apexbt.com/; Email: sales@apexbt.com.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com