Choline/Acetylcholine Quantification Colorimetric/Fluorometric 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 (λ= 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	200 µl	Red	K615-100-2A
Choline Enzyme Mix	1 Vial	Green	K615-100-4
Acetylcholinesterase	1 Vial	Blue	K615-100-5
Choline Standard (5 µmol)	1 Vial	Yellow	K615-100-6

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, 500pmol/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.

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:

   44 µl Choline Assay Buffer
   2 µl Choline Probe
   2 µl Acetylcholinesterase*
   2 µl 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).
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

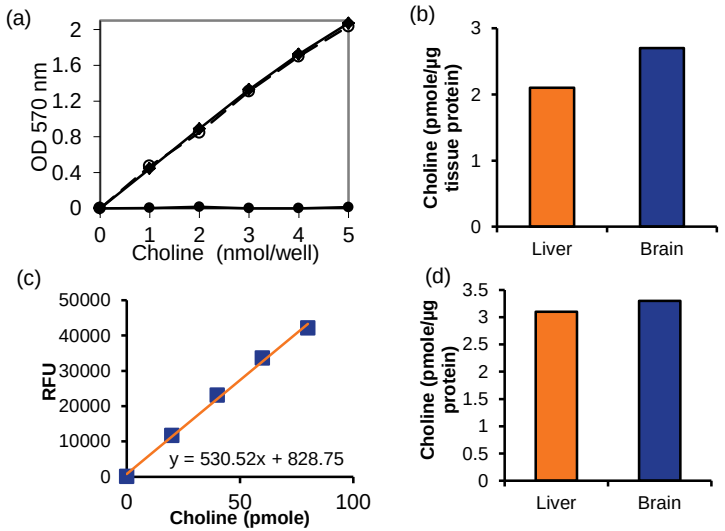

Figure: (a) Colorimetric Standard Curve. The diamonds are generated using choline as the substrate, whereas the open and closed circles were generated using acetylcholine as substrate in the presence and absence of acetylcholinesterase. (b) Choline estimation in rat liver (230 µg) and brain (150 µg) 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.

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

GENERAL TROUBLESHOOTING GUIDE:

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