Product Manual

Acetylcholine Assay Kit (Colorimetric)

Catalog Number

STA-603 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Acetylcholine is a polyatomic cation neurotransmitter that is produced in acetylcholinergic neurons. It is one of many neurotransmitters within the autonomic nervous system and the only neurotransmitter in the motor function of the somatic nervous system. Acetylcholine works within the peripheral and central nervous systems within many organisms, including humans. It is manufactured via choline acetyltransferase from acetyl-CoA and choline. Choline is an amine that is an essential nutrient that is a key precursor to many phospholipids. Acetylcholine works in the peripheral nervous system by activating skeletal muscles as well as smooth muscle and cardiac muscle function. Within the central nervous system, acetylcholine acts as a neuromodulator for the cholinergic system, which causes excitatory actions. Here the neurotransmitter is involved with plasticity, excitability, arousal, and reward. Acetylcholine's half-life and activity are very short because it is broken down by acetylcholinesterase. There are two main acetylcholine receptors: nicotinic and muscarinic.

Acetylcholine disorders can have a profound impact on neurological function. A shortage of acetylcholine, such as the autoimmune disorder Myasthenia gravis, leads to muscle fatigue and weakness due to antibodies blocking acetylcholine receptors. Acetylcholine has also been implicated in many disease states including diabetic vasculopathy, hypertension, and Alzheimer's disease.

Cell Biolabs' Acetylcholine Assay Kit is a simple colorimetric assay that measures the amount of acetylcholine 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, acetylcholine standards and samples. Sample acetylcholine concentrations are determined by comparison with a known acetylcholine standard. The kit's detection sensitivity limit is 0.75 µM acetylcholine.

Assay Principle

Cell Biolabs' Acetylcholine Assay Kit is based on the enzyme driven reaction that will detect acetylcholine via acetylcholinesterase enzyme and choline oxidase. First, acetylcholinesterase hydrolyzes acetylcholine into choline and acetic acid. Choline is then 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 acetylcholine standard within a 96-well microtiter plate format. Samples and standards are incubated for 60 minutes and then read with a standard 96-well colorimetric plate reader in the 540-570 nm range (Figure 1).

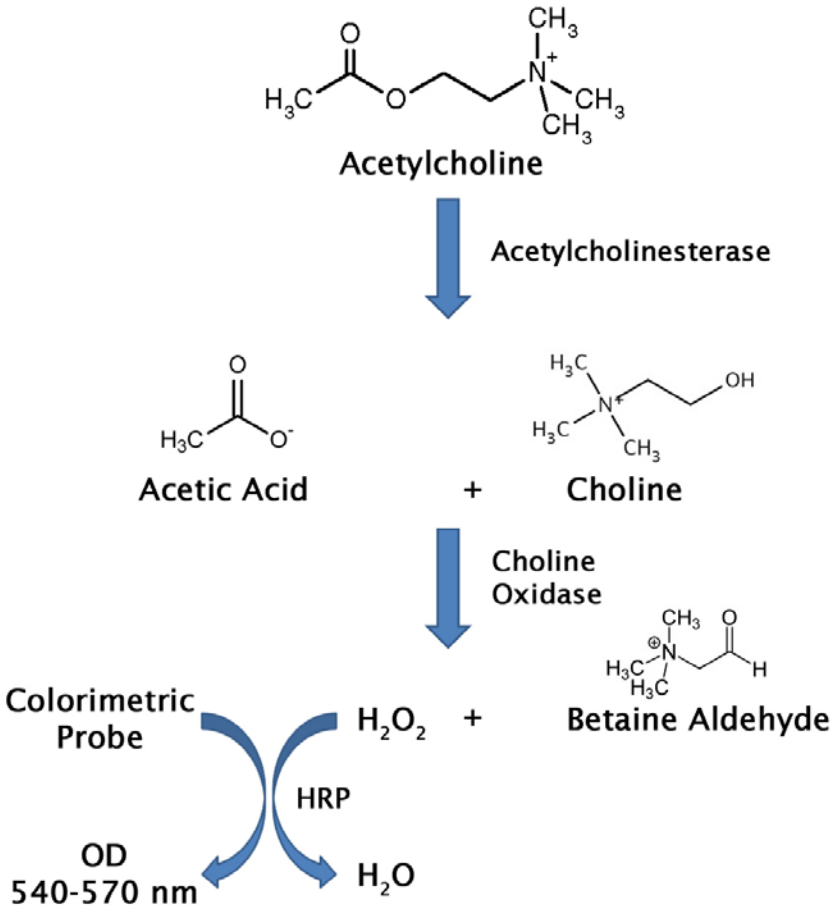

Figure 1. Colorimetric Acetylcholine Assay Principle

Related Products

1. STA-361: Human ApoAI and ApoB Duplex ELISA Kit
2. STA-368: Human ApoB-100 ELISA Kit
3. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
4. STA-384: Total Cholesterol Assay Kit (Colorimetric)
5. STA-390: Total Cholesterol Assay Kit (Fluorometric)
6. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
7. STA-396: Serum Triglyceride Quantification Kit (Colorimetric)
8. STA-600: Phosphatidylcholine Assay Kit
9. STA-601: Sphingomyelin Assay Kit
10. STA-602: Acetylcholine Assay Kit (Fluorometric)

Kit Components

Box 1 (shipped at room temperature)

1. Assay Buffer (10X) (Part No. 260202): One 50 mL bottle.
2. Colorimetric Probe (50X) (Part No. 260301): One 100 µL tube in DMSO.
3. HRP (Part No. 234402): One 100 µL tube of 100 U/mL HRP solution in glycerol.

Box 2 (shipped on blue ice packs)

1. Acetylcholine Standard (Part No. 260201): One 50 µL tube.
2. Acetylcholinesterase (Part No. 260204): One 10 Unit tube.
3. Choline Oxidase (Part No. 260205): One 25 µL tube.

Materials Not Supplied

1. 96-well microtiter plates
2. Distilled or deionized water
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. Reagents and equipment necessary for sample preparation
9. (optional) Chloroform
10. (optional) Methanol
11. (optional) Superoxide dismutase

Storage

Upon receipt, store the Assay Buffer (10X) at 4ºC. Store the remaining 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 (10X) to room temperature prior to using. Dilute the Assay Buffer (10X) with deionized water by diluting the 50 mL Buffer with 450 mL deionized water for 500 mL total. Mix to homogeneity. Store the 1X Assay Buffer at 4ºC up to six months.
- Acetylcholine Reaction Reagent: Prepare a reaction reagent to test for acetylcholine by diluting the Choline Oxidase 1:200, HRP 1:500, Colorimetric Probe 1:50, and Acetylcholinesterase 1:250 in 1X Assay Buffer. (eg. For 50 assays, combine 12.5 µL of Choline Oxidase, 5 µL of HRP, 50 µL Colorimetric Probe, and 10 µL Acetylcholinesterase with 1X Assay Buffer to 2.5 mL total

solution). Mix thoroughly and protect the solution from light. For best results, place the Acetylcholine Reaction Reagent on ice and use within 30 minutes of preparation. Do not store the Acetylcholine 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. Run proper controls as necessary. Always run a standard curve with samples.

- Tissues or Cell Suspensions: Homogenize 250 mg of sample (wet tissue or cell pellet) in 4.5 mL of chloroform/methanol (2:1, v/v). Centrifuge to remove debris. After centrifugation, incubate the homogenate at room temperature for 1 hour on an orbital shaker. Induce phase separation by adding 1.25 mL dH_2O. Incubate 10 minutes at room temperature and centrifuge at 1000 x g for 10 minutes. Collect the lower (chloroform) organic phase and re-extract the upper phase with 2 mL of solvent mixture whose composition is $CHCl_3$/MeOH/water (86:14:1, v/v/v). Combine organic phases and dry in a vacuum centrifuge. Dissolve in 200 µL $CHCl_3$/MeOH/water (60:30:4.5, v/v/v) for storage. Before acetylcholine assay, samples must be diluted at least 1:50 to 1:400 with Assay Buffer.
- 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, filter 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 serum dilutions in 1X Assay Buffer. Serum samples must be diluted at least 1:20 with Assay Buffer for accurate determinations.
- 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, filter 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. Plasma samples must be diluted at least 1:100 to 1:200 with Assay Buffer for accurate determinations.

  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).*
  3. *Choline can generate high background if present in samples. If choline may be present, run a background control without Acetylcholinesterase. Subtract this value from sample reading values.*

Preparation of Acetylcholine Standard Curve

1. Prepare fresh acetylcholine standards by first diluting a portion of the 10 mM Acetylcholine Standard stock solution 1:50 in 1X Assay Buffer. (eg. Add 10 µL of Acetylcholine Standard stock in 490 µL 1X Assay Buffer). Vortex thoroughly. This provides a 200 µM concentration. Use this 200 µM solution to prepare a series of the remaining acetylcholine standards according to Table 1 below.

Tubes	Acetylcholine Standard (µL)	1X Assay Buffer (µL)	Resulting Acetylcholine Concentration (µM)
1	10	490	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	1.57
9	250 of Tube #8	250	0.78
10	0	500	0

Table 1. Preparation of Acetylcholine Standards.

Note: Do not store diluted acetylcholine standard solutions.

Acetylcholine Assay Protocol

Each acetylcholine 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 acetylcholine standards or samples to a 96-well microtiter plate.
2. Add 50 µL of the prepared Acetylcholine 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 acetylcholine within samples by comparing the sample absorbance to the acetylcholine standard curve.

Example of Results

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

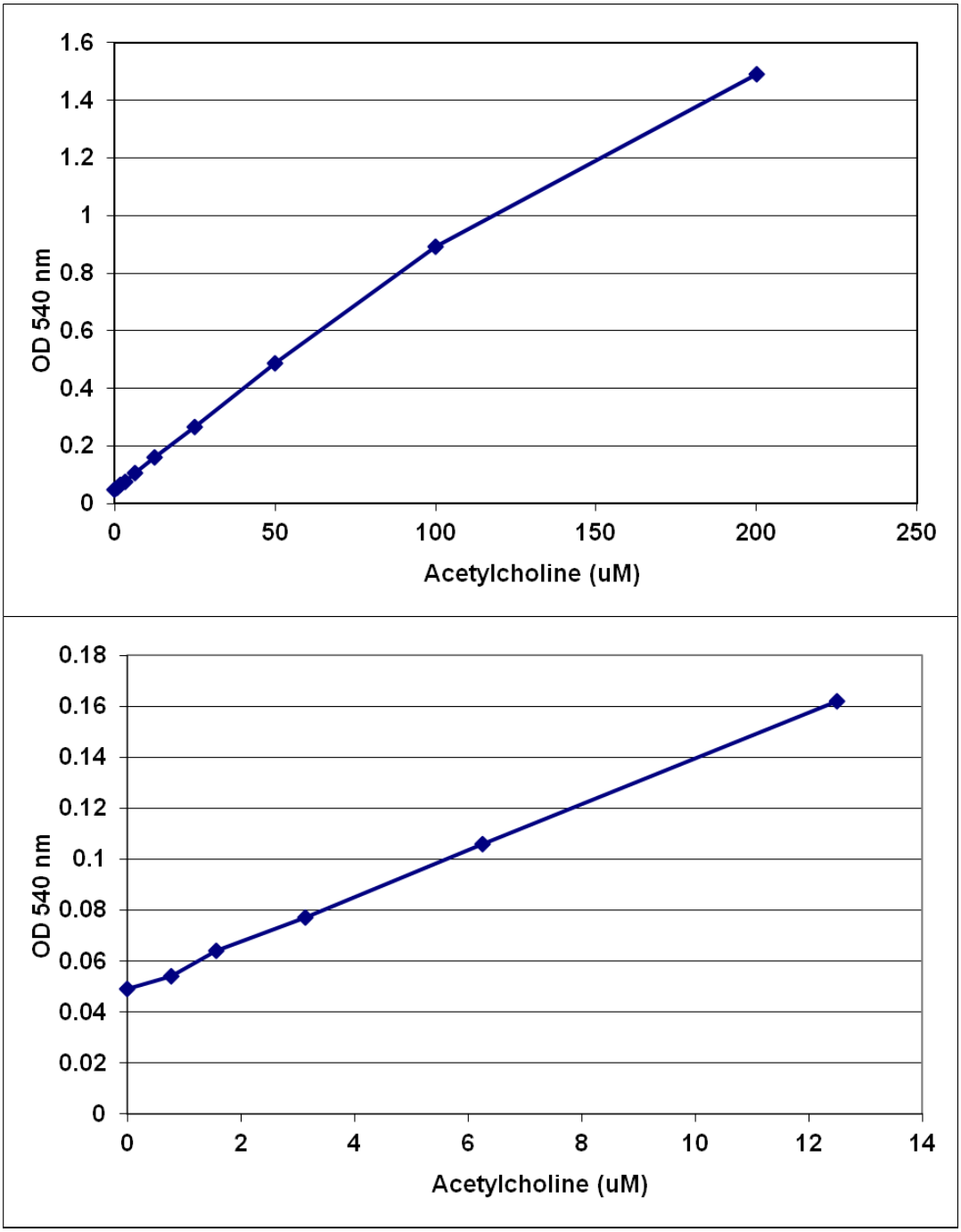

Figure 2: Acetylcholine Standard Curve.

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 acetylcholine standards from Table 1 to determine the best curve. See Figure 2 for an example standard curve.

3. Determine the acetylcholine 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.

$$\textbf{Acetylcholine (µM)} = \left[\frac{\textbf{Sample corrected absorbance}}{\textbf{Slope}} \right] \textbf{x Sample dilution}$$

Note: 1 mM acetylcholine = 14.62 mg/dL or 146 ppm.

References

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3. Kovarik, Z., et al. (2003) *Biochem. J.* **373**: 33-40.
4. Magnottl, R.A., et al. (1987) *Clin. Chem.* **33/10**: 1731-1735.
5. Vizi, E.S., et al. (1985) *J. Pharmacol. Methods* **13**: 201-211.

Warranty

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