Datasheet for ABIN2345115

Acetylcholine Assay Kit (Fluorometric)

1 Publication

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Overview

Quantity:	96 tests
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	Acetylcholine Assay Kit measures the acetylcholine present within serum, plasma, or tissue samples.
Sample Type:	Serum, Plasma
Detection Method:	Fluorometric
Sensitivity:	0.05 µM
Characteristics:	Acetylcholine Assay Kit is a simple fluorometric 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 approximately 0.05 µM acetylcholine.
Components:	1. 96-well Microtiter Plate : One 96-well clear bottom black plate. 2. Assay Buffer (10X) : Two 25 mL bottles. 3. Fluorescence Probe (50X) : One 100 µL tube in DMSO. 4. HRP : One 100 µL tube of 100 U/mL HRP solution in glycerol. Box 2 (shipped on blue ice packs)
Material not included:	1. Distilled or deionized water 2. 1X PBS

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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. Fluorescence microplate reader capable of reading excitation in the 530-570 nm range and emission in the 590-600 nm range.
7. Centrifugal filters for plasma or serum samples (e.g. Millipore Amicon Ultra-0.5 mL, Ultracel® membrane filters, or Thermo Pierce Concentrators PES membrane filters)
8. (optional) Chloroform
9. (optional) Superoxide dismutase

Target Details

Background:	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.

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Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Samples and standards are incubated and then read with a standard 96-well plate reader • Measures the amount of acetylcholine present in plasma or serum, tissue homogenates, or cell suspensions
Protocol:	The assay 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 fluorescence 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 the 96- well microtiter plate format. Samples and standards are incubated for 45 minutes and then read with a standard 96-well fluorometric plate reader .
Reagent Preparation:	1X Assay Buffer: Warm the Assay Buffer (10X) to room temperature prior to using. Dilute the Assay Buffer (10X) with deionized water by diluting one of the 25 mL bottles of buffer with 225 mL deionized water for 250 mL total. Mix to homogeneity. Store the 1X Assay Buffer at 4 °C 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, Fluorescence 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 4 Fluorescence 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.
Sample Preparation:	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. 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 dH2O. 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 CHCl3/MeOH/water (86:14:1, v/v/v). Combine organic phases and dry in a vacuum centrifuge. Dissolve in 200 µL CHCl3/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 4 °C 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 -80 °C. Prior to testing, filter samples with a 3K-10K centrifugal filter (e.g. ® Millipore Amicon Ultra-0.5 mL, Ultracel membrane filters, or Thermo Pierce

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	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. Acetylcholine levels in normal serum samples may be below the kit detection limit. Plasma: Collect blood with heparin or citrate and centrifuge at 1000 x g and 4 °C 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 -80 °C. Prior to testing, filter samples ® with a 3K-10K centrifugal filter (e.g. Millipore Amicon Ultra-0.5 mL, Ultracel membrane filters, or Thermo Pierce Concentrators PES membrane filters). Perform plasma dilutions in 1X Assay Buffer. Plasma samples must be diluted at least 1:200 to 1:400 with Assay Buffer for accurate determinations. Acetylcholine levels in normal plasma samples may be below the kit detection limit. Notes: 1. Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µ M 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 fluorescence probe is not stable in the presence of thiols (above 10 µM). 5 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.
Calculation of Results:	1. Calculate the average fluorescence 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 fluorescence. 2. Plot the corrected fluorescence 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 fluorescence values for each sample. Remember to account for dilution factors. Acetylcholine (µM) = sample corrected fluorescence x sample dilution slope Note: 1 mM acetylcholine = 14.62 mg/dL or 146 ppm. 7
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C/-20 °C
Storage Comment:	Upon receipt, store the 96-well Microtiter Plate and Assay Buffer at 4°C. Store the remaining kit

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components at -20°C. The Fluorescence Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Publications

Product cited in: Kim, Yu, Kim, Kim, Kim, Jang, Choi, Lee, Joo: "Ginsenoside Re and Rd enhance the expression of cholinergic markers and neuronal differentiation in Neuro-2a cells." in: **Biological & pharmaceutical bulletin**, Vol. 37, Issue 5, pp. 826-33, (2014) (PubMed).