EnzyChrom™ Acetylcholine Assay Kit (EACL-100)

Quantitative Colorimetric/Fluorimetric Acetylcholine Determination

DESCRIPTION

ACETYLCHOLINE is a neurotransmitter produced in acetylcholinergic neurons. It plays important roles in skeletal muscle movement, regulation of smooth and cardiac muscles, as well as in learning, memory and mood. BioAssay Systems' method provides a simple, direct and high-throughput assay for measuring acetylcholine in biological samples. In this assay, acetylcholine is hydrolyzed by acetylcholinesterase to choline which is oxidized by choline oxidase to betaine and H_2O_2. The resulting H_2O_2 reacts with a specific dye to form a pink colored product. The color intensity at 570 nm or fluorescence intensity (530/585 nm) is directly proportional to the acetylcholine concentration in the sample.

KEY FEATURES

Use 20 µL samples. Linear detection range: colorimetric assay 10 to 200 µM, fluorimetric assay 0.4 to 10 µM acetylcholine.

APPLICATIONS

Assays: acetylcholine in biological samples such as serum, plasma, urine, saliva, milk, tissue, and cell culture.

Drug Discovery/Pharmacology: effects of drugs on acetylcholine metabolism.

KIT CONTENTS

Assay Buffer: 10 mL **ACHE Enzyme:** 120 µL
Enzyme Mix: Dried **Dye Reagent:** 120 µL
Standard: 400 µL 2 mM acetylcholine

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life of six months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

COLORIMETRIC ASSAY

Sample treatment: liquid samples such as serum and plasma can be assayed directly. Tissue and cell lysates can be prepared by homogenization in cold 1 × PBS and centrifugation (5 min at 14,000 rpm). Use clear supernatants for assay. Milk samples should be cleared by mixing 600 µL milk with 100 µL 6 N HCl. Centrifuge 5 min at 14,000 rpm. Transfer 300 µL supernatant into a clean tube and neutralize with 50 µL 6 N NaOH. The neutralized supernatant is ready for assay (dilution factor *n* = 1.36).

Note: (1). SH-containing reagents (e.g. β–mercaptoethanol, dithiothreitol, > 5 µM) are known to interfere in this assay and should be avoided in sample preparation. (2). This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Working Reagent should be quick and mixing should be brief but thorough.

1. Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay. Reconstitute Enzyme Mix with 120 µL Assay Buffer. Reconstituted Enzyme Mix is stable for 1 month when stored at -20˚C. *Note: a yellow precipitate may form after thawing reconstituted Enzyme Mix. If a precipitate forms, pellet it by centrifuging for 2 min at 14000 rpm and use the clear supernatant.*

2. *Standards*: mix 24 µL 2 mM Standard with 216 µL dH_2O (final 200 µM). Dilute standard in dH_2O as follows.

No	200 µM STD + H_2O	Vol (µL)	Acetylcholine (µM)
1	100 µL + 0 µL	100	200
2	60 µL + 40 µL	100	120
3	30 µL + 70 µL	100	60
4	0 µL + 100 µL	100	0

Transfer 20 µL diluted standards into separate wells of a clear flat-bottom 96-well plate.

Samples: transfer 20 µL of each sample into separate wells of the plate. *Note: if a sample is known to contain choline, prepare an extra sample blank well with 20 µL of the sample.*

3. *Color reaction*. Prepare enough Working Reagent by mixing, for each well, 85 µL Assay Buffer, 1 µL ACHE Enzyme, 1 µL Enzyme Mix and 1 µL Dye Reagent. Add 80 µL Working Reagent to each well. *Note: for samples that contain choline, prepare a blank control reagent with no ACHE Enzyme (i.e., 85 µL Assay Buffer, 1 µL Enzyme Mix and 1 µL Dye Reagent). Add 80 µL of the control Reagent to each Sample Blank well.*

Immediately tap plate to mix. Incubate 30 min at room temperature.

4. Read optical density at 570 nm (550-585 nm).

FLUORIMETRIC ASSAY

The fluorimetric assay procedure is similar to the colorimetric procedure except that (1) 0, 3, 6 and 10 µM acetylcholine standards and (2) a black 96-well plate are used. Read fluorescence intensity at λ_{ex} = 530 nm and λ_{em} = 585 nm.

Note: if the calculated acetylcholine concentration of a sample is higher than 200 µM in the Colorimetric Assay or 10 µM in the Fluorimetric Assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor *n*.

CALCULATION

Subtract blank value (#4) from the standard values and plot the ΔOD or ΔF against standard concentrations. Determine the slope and calculate the acetylcholine concentration of Sample,

$$[\text{Acetylcholine}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \qquad (\mu M)$$

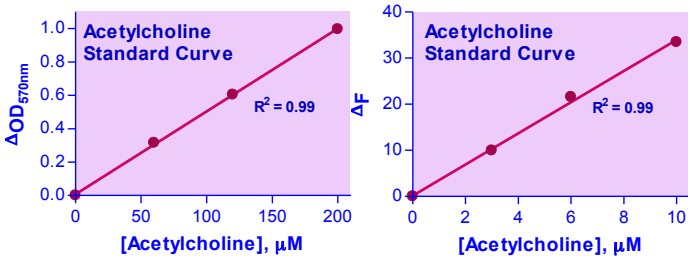
R_{SAMPLE} and R_{BLANK} are optical density or fluorescence intensity readings of the Sample and H_2O Blank (or Sample Blank if sample contains choline), respectively. *n* is the sample dilution factor.

Conversions: 1 mM acetylcholine equals 14.6 mg/dL, 0.015% or 146 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates, optical density plate reader; black flat-bottom uncoated 96-well plates, fluorescence plate reader.

Acetylcholine Standard Curves



96-well colorimetric assay *96-well fluorimetric assay*

LITERATURE

1. Vizi, E.S. et al (1985). A simple and sensitive method of acetylcholine identification and assay. Bioassay combined with minicolumn gel filtration or high-performance liquid chromatography. J Pharmacol Methods. 13:201-211.
2. Gilberstadt, M.L. and Russell, J.A. (1984). Determination of picomole quantities of acetylcholine and acetylcholine in physiologic salt solutions. Anal Biochem. 138:78-85.
3. Israel, M. and Lesbats, B. (1982). Application to mammalian tissues of the chemiluminescent method for detecting acetylcholine. J Neurochem. 39:248-250.