

## 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<sub>2</sub>O<sub>2</sub>. The resulting H<sub>2</sub>O<sub>2</sub> reacts with a specific dye to form a pink colored product. The color intensity at 570nm 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:** 120 µL                      **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 three 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 x 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.
2. **Standards:** mix 24 µL 2 mM Standard with 216 µL dH<sub>2</sub>O (final 200 µM). Dilute standard in dH<sub>2</sub>O as follows.

No	200 µM STD + H <sub>2</sub> O	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 20 min at room temperature.

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

#### 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  $\lambda_{ex} = 530$  nm and  $\lambda_{em} = 585$  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  $\Delta OD$  or  $\Delta F$  against standard concentrations. Determine the slope and calculate the acetylcholine concentration of Sample,

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

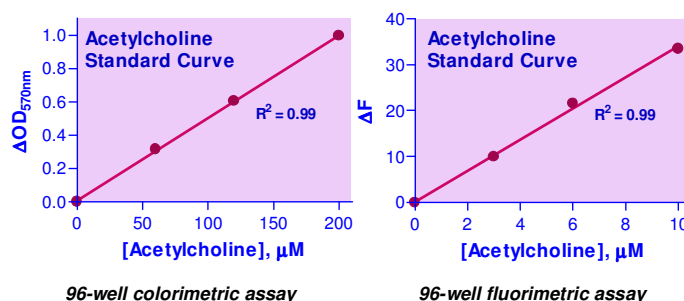
$R_{\text{SAMPLE}}$  and  $R_{\text{BLANK}}$  are optical density or fluorescence intensity readings of the Sample and H<sub>2</sub>O Blank (or Sample Blank if sample contains choline), respectively.  $n$  is the sample dilution factor.

**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.

#### Choline Standard Curves



#### 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.