

## 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 570 nm or fluorescence intensity (530/585 nm) is directly proportional to the acetylcholine concentration in the sample.

#### KEY FEATURES

Use 20  $\mu$ L samples. Linear detection range: colorimetric assay 10 to 200  $\mu$ M, fluorimetric assay 0.4 to 10  $\mu$ M 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  $\mu$ L  
**Enzyme Mix:** Dried                      **Dye Reagent:** 120  $\mu$ L  
**Standard:** 400  $\mu$ L 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  $\times$  PBS and centrifugation (5 min at 14,000 rpm). Use clear supernatants for assay. Milk samples should be cleared by mixing 600  $\mu$ L milk with 100  $\mu$ L 6 N HCl. Centrifuge 5 min at 14,000 rpm. Transfer 300  $\mu$ L supernatant into a clean tube and neutralize with 50  $\mu$ L 6 N NaOH. The neutralized supernatant is ready for assay (dilution factor  $n = 1.36$ ).

**Note:** (1). *SH-containing reagents (e.g.  $\beta$ -mercaptoethanol, dithiothreitol, > 5  $\mu$ M) 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.*

- Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay. Reconstitute Enzyme Mix with 120  $\mu$ L 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.*
- Standards:** mix 24  $\mu$ L 2 mM Standard with 216  $\mu$ L dH<sub>2</sub>O (final 200  $\mu$ M). Dilute standard in dH<sub>2</sub>O as follows.

No	200 $\mu$ M STD + H <sub>2</sub> O	Vol ( $\mu$ L)	Acetylcholine ( $\mu$ M)
1	100 $\mu$ L + 0 $\mu$ L	100	200
2	60 $\mu$ L + 40 $\mu$ L	100	120
3	30 $\mu$ L + 70 $\mu$ L	100	60
4	0 $\mu$ L + 100 $\mu$ L	100	0

Transfer 20  $\mu$ L diluted standards into separate wells of a clear flat-bottom 96-well plate.

**Samples:** transfer 20  $\mu$ L 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  $\mu$ L of the sample.*

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

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

- 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  $\mu$ M 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  $\mu$ M in the Colorimetric Assay or 10  $\mu$ M 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,

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

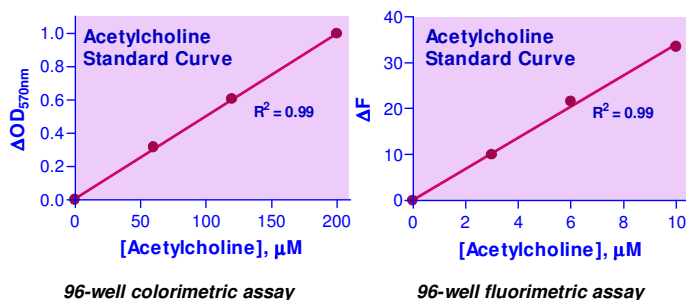
$R_{\text{SAMPLE}}$  and  $R_{\text{BLANK}}$  are optical density or fluorescence intensity readings of the Sample and H<sub>2</sub>O Blank (or Sample Blank if sample contains choline), respectively.  $n$  is the sample dilution factor.

**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.

#### Acetylcholine Standard Curves



#### PUBLICATIONS

- He C, et al (2012). Embryonic exposure to benzo(a)pyrene influences neural development and function in rockfish (*Sebastes marmoratus*). *Neurotoxicology* 33(4):758-62.
- Chen L et al (2012). Acute exposure to DE-71: Effects on locomotor behavior and developmental neurotoxicity in zebrafish larvae. *Environ Toxicol Chem.* 31(10):2338-44.
- Baitharu, I., et al (2013). Withania somnifera root extract ameliorates hypobaric hypoxia induced memory impairment in rats. *Journal of ethnopharmacology* 145(2): 431-441.

