

Technical Manual

ColorFluor Acetylcholine Assay Kit

Catalogue Code: BA0069

Pack Size: 100 assays

Research Use Only

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. The Assay Genie ColorFluor Acetylcholine Assay Kit 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 H₂O₂. The resulting H₂O₂ 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 μ L samples. Linear detection range: colorimetric assay 10 to 200 μ M, fluorimetric assay 0.4 to 10 μ 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 μ L
Enzyme Mix: Dried **Dye Reagent:** 120 μ L
Standard: 400 μ 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 x PBS and centrifugation (5 min at 14,000 rpm). Use clear supernatants for assay. Milk samples should be cleared by mixing 600 μ L milk with 100 μ L 6 N HCl. Centrifuge 5 min at 14,000 rpm. Transfer 300 μ L supernatant into a clean tube and neutralize with 50 μ L 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 μ 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.

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 μ L Assay Buffer. Reconstituted Enzyme Mix is stable for 1 month when stored at -20°C.
2. **Standards:** mix 24 μ L 2 mM Standard with 216 μ L dH₂O (final 200 μ M). Dilute standard in dH₂O as follows.

No	200 μ M STD + H ₂ O	Vol (μ L)	Acetylcholine (μ M)
1	100 μ L + 0 μ L	100	200
2	60 μ L + 40 μ L	100	120
3	30 μ L + 70 μ L	100	60
4	0 μ L +100 μ L	100	0

Transfer 20 μ L 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 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 $\lambda_{\text{ex}} = 530 \text{ nm}$ and $\lambda_{\text{em}} = 585 \text{ nm}$.

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,

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

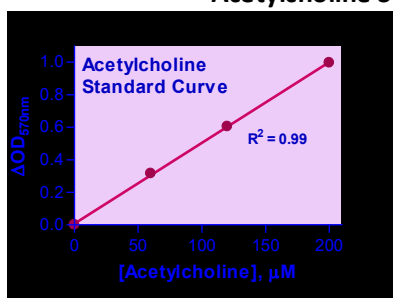
R_{SAMPLE} and R_{BLANK} are optical density or fluorescence intensity readings of the Sample and H_2O Blank (or Sample Blank if sample contains choline), respectively. n is the sample dilution factor.

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

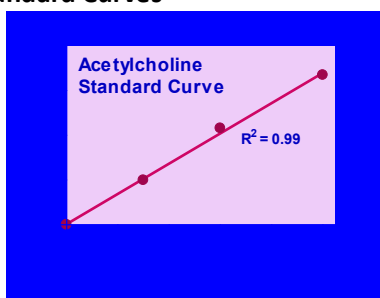
MATERIALS REQUIRED, BUT NOT PROVIDED

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.

Contact Details

Dublin, Ireland

Email: info@assaygenie.com

Web: www.assaygenie.com

Technical Support: Techsupport@assaygenie.com