Choline Assay Kit (Colorimetric/Fluorometric)

LS-K260-100 (100 Tests) • **Store at -20°C**

Introduction

CHOLINE and its metabolites play important roles in membrane structure integrity, cellular signaling and cholinergic neurotransmission. Aberrant regulation in choline metabolism has been associated with mental illness such as anxiety. LSBio's' method provides a simple, direct and high-throughput assay for measuring choline in biological samples. In this assay, free choline is oxidized by choline oxidase to betaine and H_2O_2 which 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 choline concentration in the sample.

Key Features

- Use 20 µL samples. Linear detection range: colorimetric assay 1 to 100 µM, fluorometric assay 0.2 to 10 µM choline.

Applications

- Assays: choline in biological samples such as serum, plasma, urine, saliva, milk, tissue, and cell culture.
- Drug Discovery/Pharmacology: effects of drugs on choline metabolism.

Components

Component	K260-100
	100 Tests
Assay Buffer	10 mL
Enzyme Mix	Dried
Dye Reagent	120 µL
Standard (2 mM Choline)	400 µL

Materials Not Supplied

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.

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

Assay Procedure

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 14,000rpm). 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).

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121
www.LSBio.com • (206) 464-1554 • TechnicalSupport@LSBio.com

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 enzymecatalyzed 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 12 µL 2 mM Standard with 228 µL dH_2O (final 100 µM). Dilute standard in dH_2O as follows.

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

Transfer 20 µL diluted standards into separate wells of a clear flatbottom 96-well plate. Samples: transfer 20 µL of each sample into separate wells of the plate.

3. Color reaction. Prepare enough Working Reagent by mixing, for each reaction well, 85 µL Assay Buffer, 1 µL Enzyme Mix and 1 µL Dye Reagent. Add 80 µL Working Reagent to each well. Tap plate to mix. Incubate 30 min at room temperature.
4. Read optical density at 570 nm (550-585 nm).

Fluorometric Assay

The fluorometric assay is 10 times more sensitive than the colorimetric method. The procedure is similar to that for the Colorimetric Assay except that (1) 0, 3, 6 and 10 µM choline standards and (2) a black 96-well plate are used. Read fluorescence intensity at λex = 530 nm and λem = 585 nm.

Note: if the calculated choline concentration of a sample is higher than 100 µM in the Colorimetric Assay or 10 µM in the Fluorometric Assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor *n*.

Calculations

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

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

R_{SAMPLE} and R_{BLANK} are optical density or fluorescence intensity readings of the Sample and H2O Blank, respectively. *n* is the sample dilution factor.

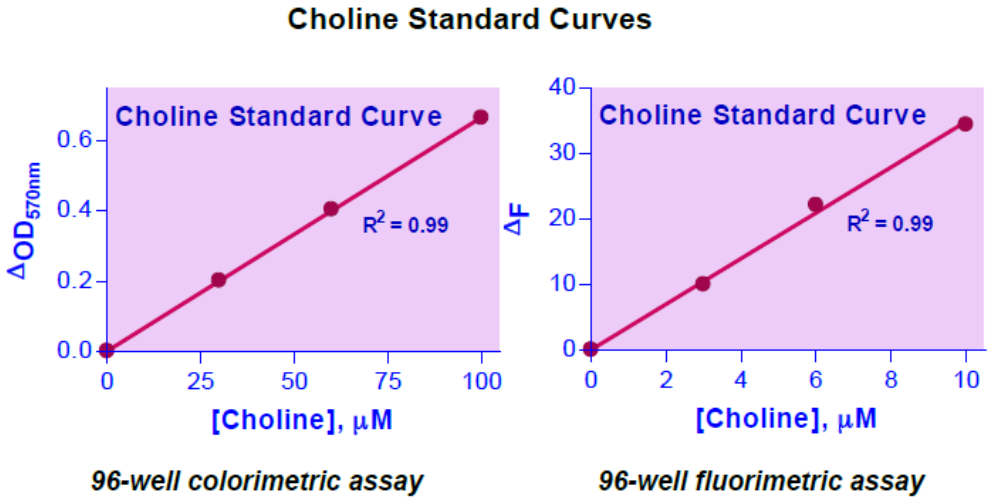
Conversions: 1 mM choline equals 10.4 mg/dL, 0.010% or 104 ppm.

Choline Assay Kit (Colorimetric/Fluorometric)

LS-K260-100 (100 Tests) • **Store at -20°C**

LSBio
LifeSpan BioSciences, Inc.

Sample Data



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

Version: V.08.09.2018