USER GUIDE

**For Research Use Only. Not For Use In Diagnostic Procedures**

**Version 2.0**

# 胆碱检测试剂盒

# Choline Assay Kit

**Do not eat Store at -20° C & in the dark.**

**Cat.No.**MCE9756 **Size :** 100 tests

**Technical literature is available at :** www.mesgenbio.com
**E-mail MesGen Technical Services if you have questions on use of this system :** tech@mesgenbio.com

## Description

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. MesGen Biotechnology' 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 0.6 to 150 µM, fluorimetric assay 0.1 to 15 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.

## Kit Contents

**Reagent A:** 1.6 mL **Reagent B:** 1.6 mL **Reagent C:** 120 µL

**Assay Buffer:** 10 mL

**Standard**: 500 µL 2 mM Choline

## 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 1XPBS 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 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.
2. *Standards*: mix 20 µL 2 mM Standard with 180 µL $dH_2O$ (final 200 µM). Dilute standard in $dH_2O$ as follows.

| No. | 200 µM STD + $H_2O$ | Vol (µL) | Choline (µM) |
|---|---|---|---|
| 1 | 75 µL + 25 µL | 100 | 150 |
| 2 | 50 µL + 50 µL | 100 | 100 |
| 3 | 25 µL + 75 µL | 100 | 50 |
| 4 | 10 µL + 90 µL | 100 | 20 |
| 5 | 5 µL + 95 µL | 100 | 10 |
| 6 | 1 µL + 99 µL | 100 | 2 |
| 7 | 0.5 µL + 99.5 µL | 100 | 1 |
| 8 | 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 r*eaction*. Prepare enough Working Reagent by mixing, for each reaction well, 70 µL Assay Buffer, 15 µL Reagent A, 15 µL Reagent B, 1 µL Reagent C. Add 100 µL Working Reagent to each well. Tap plate to mix. Incubate 5-10 min at room temperature.
4. Read optical density at 570 nm (550-585 nm).

## Fluorimetric Assay

The fluorimetric assay is about 10 times more sensitive than the colorimetric method. The procedure is similar to that for the Colorimetric Assay except that (1) 0, 1, 5,10 and 15 µM choline 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 choline concentration of a sample is higher than 150 µM in the Colorimetric Assay or 15 µM in the Fluorimetric Assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor *n*.

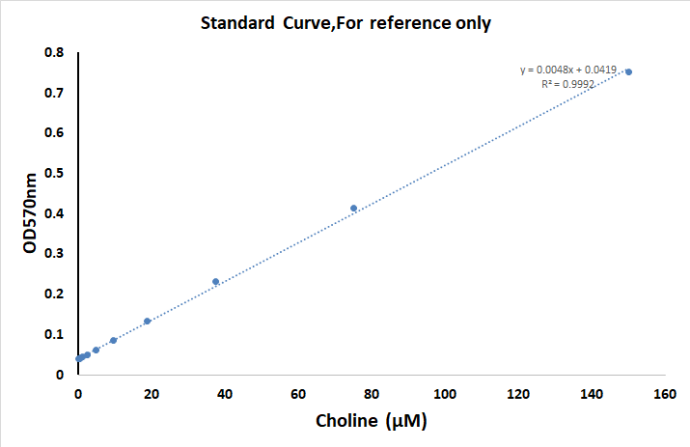
## Calculation

Subtract blank value 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 $H_2O$ Blank, respectively. $n$ is the sample dilution factor.

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




## Storage conditions

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

## Shelf life

6 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.

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