Technical Bulletin

Choline Assay Kit

Catalogue number MAK508

Product Description

Choline and its metabolites play an important role in membrane structure integrity, cellular signaling and cholinergic neurotransmission. Aberrant regulation in choline metabolism has been associated with mental illness such as anxiety. The Choline Assay kit 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 at λ_{Ex} = 530 nm/λ_{Em} = 585 nm is directly proportional to the choline concentration in the sample.

The linear detection range of the kit is 1 to 100 μM choline for colorimetric assays and 0.2 to 10 μM for fluorometric assays. The kit is suitable for choline determination in biological samples such as serum, plasma, urine, saliva, milk, tissue, and cell culture, as well as for studying the effects of drugs on choline metabolism.

Components

The kit is sufficient for 100 colorimetric or fluorometric assays in 96-well plates.

- Assay Buffer — 10 mL
  Catalogue Number MAK508A
- Enzyme Mix — 1 vial
  Catalogue Number MAK508B
- Dye reagent — 120 μL
  Catalogue Number MAK508C
- Standard (2 mM Choline) — 400 μL
  Catalogue Number MAK508D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example multichannel pipettor)
- Multiwell plate reader.
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL centrifuge tubes.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

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

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Enzyme mix: Reconstitute Enzyme Mix with 120 μL Assay Buffer. Reconstituted Enzyme Mix is stable for 1 month when stored at -20 °C. Keep thawed tubes on ice during assay.

Note:

1. Yellow precipitate may form after thawing reconstituted Enzyme Mix. If a precipitate forms, pellet it by centrifuging for 2 minutes at 14000 rpm and use the clear supernatant.
2. This assay is based on an enzyme catalyzed kinetic reaction. The addition of a Working Reagent should be quick, and mixing should be brief but thorough.

Procedure

Sample Preparation

Note: SH-containing reagents (for example β-mercaptoethanol, dithiothreitol, >5 μM) are known to interfere in this assay and should be avoided in Sample preparation.

1. Liquid Samples such as serum and plasma can be assayed directly.
2. Tissue and cell (10^6-10^7) lysates can be prepared by homogenization in cold 1 × PBS and centrifuge at 14,000 rpm for 5 minutes. Use clear supernatant for assay.
3. Milk Samples should be cleared by mixing 600 μL milk with 100 μL of 6 N HCl. Centrifuge mixture for 5 minutes at 14,000 rpm. Transfer 300 μL supernatant into a clean tube and neutralize with 50 μL of 6 N NaOH. The neutralized supernatant is ready for assay (dilution factor n = 1.36)

Transfer 20 μL of each Sample into separate wells of the plate.

Colorimetric Standard Curve Preparation

1. Prepare 100 μM Standard by diluting 12 μL of 2 mM standard with 228 μL purified water.
2. Dilute Standard in purified water as mentioned in Table 1.

Table 1.
Preparation of Colorimetric Choline Standards

Well No.	100 μM Standard	Purified Water	Choline (μM)
1	100 μL	0 μL	100
2	60 μL	40 μL	60
3	30 μL	70 μL	30
4	0 μL	100 μL	0

3. Transfer 20 μL Standards into separate wells of a clear flat-bottom 96-well plate.

Fluorometric Standard Curve Preparation

1. Prepare Standards according to Colorimetric Standard Curve Preparation section.
2. Mix 10 μL of the Standards from Colorimetric Procedure with 90 μL of purified water according to Table 2.

Table 2.
Preparation of Choline Standards

Well No.	Colorimetric Standard	Assay Buffer	Choline (μM)
1	10 μL of 100 μM Std	90 μL	10
2	10 μL of 60 μM Std	90 μL	6
3	10 μL of 30 μM Std	90 μL	3
4	-	100μL	0

3. Transfer 20 μL Standards separate wells of a black 96-well plate.

Working Reagent Preparation

Mix enough reagents for the number of assays to be performed. For each Sample and Standard well, according to Table 3.

Table 3.
Preparation of Working Reagent

Reagent	Volume
Assay Buffer	85 μL
Enzyme mix	1 μL
Dye reagent	1 μL

Transfer 80 μL of Working Reagent into each Sample and Standard well. Tap plate to mix.

Measurement

1. Incubate the plate for 30 minutes at room temperature.
2. Measure the optical density at 570 nm for colorimetric assay or fluorescence intensity at λ_{Ex} = 530 nm/λ_{Em} = 585 nm.

Results

1. Calculate ΔOD or ΔF by subtracting the reading (OD or fluorescence intensity F) of Standard #4 (Blank) from the remaining Standard reading values.
2. Plot the ΔF or ΔOD against the standard concentrations and determine the slope of the Standard curve.
3. Calculate the Choline concentration of samples using the below equation:

$$[\text{Choline}] = \frac{R_{\text{Sample}} - R_{\text{Blank}}}{\text{Slope}(\mu M^{-1})} \times n \quad (\mu M)$$

Where:

R_{SAMPLE} = OD or fluorescence intensity (F) reading of Sample

R_{BLANK} = OD or fluorescence intensity (F) reading of Blank

DF = Sample dilution factor (DF = 1 for undiluted Samples)

Note: If the calculated Choline concentration of a sample is higher than 100 μM for the colorimetric assay or 10 μM in the fluorometric assay, dilute sample in purified water and repeat the assay. Multiply result by the dilution factor (DF).

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

Figure 1.

Typical Colorimetric Choline Standard Curve

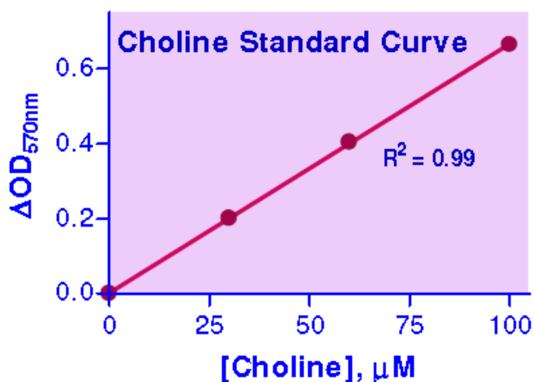
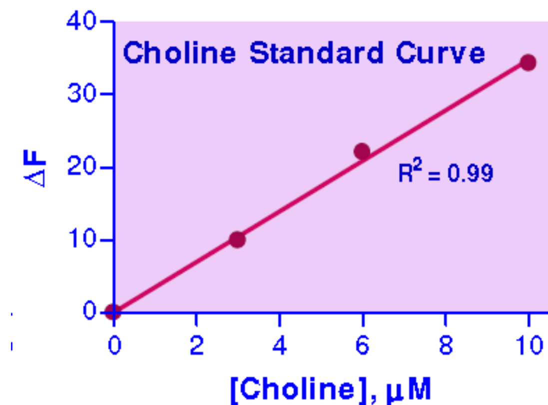


Figure 2.

Typical Fluorometric Choline Standard Curve



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