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Product Information

Choline/Acetylcholine Quantitation Kit

Catalog Number **MAK056**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Choline is an essential nutrient, commonly grouped with the B complex vitamins, that plays key roles in many biological processes. Choline is a precursor for the synthesis of acetylcholine, a critical neurotransmitter in both the peripheral and central nervous systems.

In this assay, choline concentration is determined by a coupled enzyme reaction, which results in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product, proportional to the choline present. Acetylcholine levels can be determined by adding acetylcholinesterase to the reaction, which hydrolyzes acetylcholine to choline and acetate. This kit is suitable for use with cell and tissue culture supernatants as well as serum, plasma, urine, and other biological fluids.

Components

The kit is sufficient for 100 assays in 96 well plates.

Choline Assay Buffer Catalog Number MAK056A	25 mL
Choline Probe, in DMSO Catalog Number MAK056B	0.2 mL
Choline Enzyme Mix Catalog Number MAK056C	1 vl
Acetylcholinesterase Catalog Number MAK056D	1 vl
Choline Standard, 5 µmole Catalog Number MAK056E	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Choline Assay Buffer – Allow buffer to come to room temperature before use.

Choline Probe – Thaw at room temperature to melt the solution prior to use. Aliquot and store protected from light and moisture at –20 °C. Upon thawing, the Choline Probe is ready-to-use in the colorimetric assay.

For the fluorescence assay, dilute an aliquot of the Choline Probe Solution 5 to 10-fold with Choline Assay Buffer, just prior to use. This will reduce the background of the fluorescence assay.

Choline Enzyme Mix, Acetylcholinesterase – Reconstitute each with 220 µL of Choline Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

Choline Standard – Reconstitute in 100 µL of Choline Assay Buffer to generate a 50 mM (50 nmole/µL) Choline Standard solution. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution and keep cold while in use.

Storage/Stability

The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Choline Standards for Colorimetric Detection
Dilute 10 µL of the 50 mM (50 nmole/µL) Choline Standard Solution with 990 µL of Choline Assay Buffer to prepare a 0.5 mM (0.5 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.5 mM Choline standard solution into a 96 well plate, generating 0 (blank), 1, 2, 3, 4, and 5 nmole/well standards. Add Choline Assay Buffer to each well to bring the volume to 50 µL.

Choline Standards for Fluorometric Detection
Dilute 10 µL of the 50 mM (50 nmole/µL) Choline Standard Solution with 990 µL of Choline Assay Buffer to prepare a 0.5 mM (0.5 nmole/µL) standard solution. Dilute 10 µL of the 0.5 mM standard solution with 90 µL of Choline Assay Buffer to generate a 0.05 mM (50 pmole/µL). Add 0, 2, 4, 6, 8, and 10 µL of the 0.05 mM Choline standard solution into a 96 well plate, generating 0 (blank), 100, 200, 300, 400, and 500 pmole/well standards. Add Choline Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (10 mg) or cells (2×10^6) should be rapidly homogenized in 4 volumes of cold Choline Assay buffer. Centrifuge at 13,000 × *g* for 10 minutes at 4 °C to remove insoluble material.

Serum samples can be added directly to the wells.

Bring samples to a final volume of 50 µL with Choline Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Free choline	Total Choline
Choline Assay Buffer	46 µL	44 µL
Choline Probe	2 µL	2 µL
Acetylcholinesterase	–	2 µL
Enzyme Mix	2 µL	2 µL

Notes: To determine free choline only, omit the acetylcholinesterase from the reaction and add 46 µL of the Choline Assay Buffer to the reaction mix. Acetylcholinesterase converts acetylcholine to choline. In the presence of acetylcholinesterase, this enzyme detects total choline (free choline plus acetylcholine). To determine acetylcholine, the sample should be separately run in duplicate with both Reaction Mixes.

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at room temperature. Protect the plate from light during the incubation.

3. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Choline Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Choline standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Concentration of Choline

Determination of free Choline using Reaction Mix without acetylcholinesterase

$$S_a/S_v = C$$

S_a = Amount of free Choline in unknown sample (nmole) from standard curve
S_v = Sample volume (µL) added into the wells
C = Concentration of Choline in sample

Choline molecular weight: 104.2 g/mole

Sample Calculation

Amount of Choline (S_a) = 3.84 nmole (from standard curve)
Sample volume (S_v) = 50 µL

Concentration of Choline in sample

$$3.84 \text{ nmole}/50 \text{ µL} = 0.077 \text{ nmole}/\text{µL}$$

$$0.077 \text{ nmole}/\text{µL} \times 104.2 \text{ ng/nmole} = 8.02 \text{ ng}/\text{µL}$$

Concentration of Acetylcholine

Requires determination of free Choline (Reaction Mix without acetylcholinesterase) and total Choline (Reaction Mix with acetylcholinesterase).

Acetylcholine (S_{acetyl}) = total choline (S_{total}) – free choline (S_{free})

$$S_{acetyl} = S_{total} - S_{free}$$

S_{total} = Amount of total Choline in unknown sample (nmole) from standard curve (assay with acetylcholinesterase)
S_{free} = Amount of free Choline in unknown sample (nmole) from standard curve (assay without acetylcholinesterase)

$$S_{acetyl}/S_v = C$$

S_v = Sample volume (µL) added into the wells
C = Concentration of Acetylcholine in sample

Acetylcholine molecular weight: 146.2 g/mole

Sample Calculation

Amount of total Choline (S_{total}) = 4.96 nmole (from standard curve)
Amount of free Choline (S_{free}) = 3.84 nmole (from standard curve)
Amount of Acetylcholine (S_{acetyl}) = 1.12 nmole ($S_{acetyl} = S_{total} - S_{free}$)
Sample volume (S_v) = 50 µL

Concentration of Acetylcholine in sample

$$1.12 \text{ nmole}/50 \text{ µL} = 0.022 \text{ nmole}/\text{µL}$$

$$0.022 \text{ nmole}/\text{µL} \times 146.2 \text{ g/mole} = 3.22 \text{ ng}/\text{µL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice Cold Assay Buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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