MA-0152

Choline/Acetylcholine Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Introduction:

Choline plays a vital role in various metabolic processes. As a precursor to acetylcholine, it's crucial for maintaining healthy neurological function. Choline is involved in lipid metabolism both aiding in the transport and metabolism of fats in the liver and as a precursor to phosphatidylcholine, a major component of membranes. Choline is also highly important as a methyl donor, contributing to the formation of S-adenosylmethionine, a molecule with diverse functions. Small amounts of choline can be synthesized but dietary intake is important to maintain the amounts required for optimal health. AkrivisBio's Choline Assay is a simple sensitive method of measuring choline in a variety of biological samples with a sensitivity from the low picomole to the multi-nanomole range.

Assay Principle:

1 – Choline is oxidized by Choline Oxidase forming glycine betaine and hydrogen peroxide.
2 – Hydrogen peroxide is utilized by peroxidase as an electron acceptor to oxidize ADHP to resorufin with a strong increase in color and fluorescence.

Assay Components:

Assay Buffer	25 ml	WM	MA-0152A
ADHP Solution	200 µl	Red	MA-0152B
Choline Oxidase/Peroxidase	lyoph	Green	MA-0152C
Acetylcholinesterase	lyoph	Blue	MA-0152D
Choline Standard (5 µmol)	lyoph	Yellow	MA-0152E

Storage and Handling Considerations:

Keep unopened Assay at -20°C. Warm all components to room temperature when preparing to use the assay.
Assay Buffer: Ready to use as supplied. Store at 4°C.
ADHP Solution: Make sure DMSO is completely melted before use. Light and water sensitive. Store at -20°C.
Choline Oxidase/Peroxidase, Acetylcholinesterase: Reconstitute with 220 µl of Assay Buffer. It is advisable to aliquot into convenient portions and keep at -20°C to avoid repeated freeze/thaw cycles.
Choline Standard: Reconstitute with 1 ml µl of DI water making a 5 mM solution.

Assay Protocol:

1. Standard Curve:
Absorbance-based Assay: Transfer 10 µl of the standard into 240 µl of Assay Buffer. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl to a series of wells in a 96-well plate. Adjust all well volumes to 50 µl with Assay Buffer giving 0 – 1 – 2 – 3 – 4 – 5 nmoles of the choline standard.

Fluorescence-based Assay: Dilute the choline standard as for the absorbance-based assay, then dilute it 10X more, transferring 50 µl of diluted choline standard to 450 µl of Assay Buffer. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl to a series of wells and adjust all well volumes to 50 µl with Assay Buffer giving 0 – 100 – 200 – 300 – 400 – 500 pmoles of the standard. For acetylcholine assays, where tissue concentrations are quite low (~1 – 10 nmol/g tissue outside of the brain), a more limited standard curve can be created with a range of 0 – 50 or less pmoles. Just dilute the standard appropriately for the range you desire.

2. Sample Preparation: Liquid samples can be applied directly to a 96-well plate. Serum contains ~ 10 µM choline, so sample size on the order of 10-20 µl is sufficient. Acetylcholine in tissue is low. Homogenize 25- 50 mg of tissue or cells in 100-200 µl Assay Buffer then centrifuge at 16,000 X g to remove debris. Transfer the clear supernatant to a fresh tube. Samples should be fresh. Choline levels will increase in stored samples due to lipid breakdown.

3. Reaction Mix: Each sample and standard well requires 50 µl of Reaction Mix. Prepare sufficient material for the number of wells to be analyzed, containing:

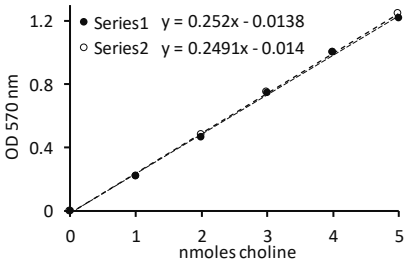
	Reaction Mix	
Assay Buffer	44 µl	
ADHP Solution:	2 µl	
Acetylcholinesterase	2 µl	Omit Acetylcholinesterase if free choline only is to be measured.
Choline Oxidase/Peroxidase	2 µl	

Add 50 µl of Reaction Mix to each well containing Standards or test samples, mix well.

4. Measure: Read the wells in a plate reader at 570 nm (color) or 535/587 nm (fluorescence) at room temperature for 30 minutes.

5. Typical Results:

	Standard Raw Values		Background Corrected Values	
nmole Standard	OD			
0	0.0559	0.0541	0	0
1	0.2759	0.2752	0.2200	0.2211
2	0.5195	0.5368	0.4636	0.4827
3	0.8055	0.8056	0.7496	0.7515
4	1.0574	1.0518	1.0015	0.9977
5	1.2735	1.2987	1.2176	1.2446



6. Calculations: Subtract the zero choline standard from all other standards and sample readings. Plot the Standard Curve. Determine the slope of the Standard Curve. This value defines the sensitivity of the measurement system. Divide the background corrected samples readings by the slope of the Standard Curve to obtain nmoles or pmoles of choline in the sample wells. To convert this value back to the amount of choline in the original samples:

A Divide the amount of choline in the sample wells by the volume of sample applied to the wells = nmoles choline per µl of sample.
B Multiply nmoles choline per µl of sample by the total volume of the clear supernatant obtained in Step 2 above = total choline per sample.
C Divide total choline per sample by mg of tissue or # of cells used as sample = amount of choline per mg tissue (or per # of cells, etc.)
Liquid samples need only be corrected for any dilution factors used in preparing the samples.

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