Choline Assay (Cho)

Cat. No. 8588
100 Tests in 96-well plate

Introduction

Choline is an essential nutrient and plays a key role in many biological processes. Choline and its metabolites are needed for various physiological purposes, including: structural integrity of cell membranes, cell membrane signaling, cholinergic neurotransmission, and additionally, they are a major source of methyl groups that are needed for the biosynthesis of lipids and the regulation of metabolic pathways. Choline deficiency plays a role in liver disease, atherosclerosis, neurological disorders, and organ dysfunction. This colorimetric assay is based on choline oxidase-catalyzed oxidation of free choline, where the formed hydrogen peroxide is then catalyzed by peroxidase and reacts with 4-aminoantipyrine to form a colored product that can be read at 550nm. The intensity of the colored reaction product is directly proportional to the concentration of choline in the sample.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8588a	1	Assay buffer	25 mL	4°C
8588b	1	Choline standard	1.0 mL	-20°C
8588c	1	Substrate mix	1.6 mL	-20°C
8588d	1	Enzyme mix	0.2 mL	-20°C

Product Use

The Choline Assay kit measures choline level in various types of samples, including cells, serum, plasma, and tissue. This product is for research purposes only and is not approved for use in animals, humans, or diagnostic procedures.

Quality Control

Data from the Choline Assay kit using choline solutions with concentrations ranging from 6.25-400 µM show a linear relationship between OD_{550nm} and choline concentration (Figure 1).

Shipping

Shipped on dry ice.

Procedures (96-well plate)

A. Preparation of choline standard

1. Add 60 µL of choline standard (8588b) to 15 µL of assay buffer (8588a) to make a 75 µL solution of 800 µM choline.
2. Obtain 8 test tubes, add 50 µL of assay buffer (8588a) into each tube and label them #1 through #8.
3. Add 50 µL of the 800 µM choline solution into tube #1 and mix well to obtain the 400 µM choline standard.
4. Transfer 50 µL of the 400 µM choline standard from tube #1 to tube #2 and mix well to obtain the 200 µM choline standard.
5. Repeat step 4 for tubes #3-7 to serially dilute the choline standards. Do not add any choline to tube #8, which serves as a blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A and B) of each choline standard by aliquoting 20 µL/well of each choline standard into duplicate wells of the 96-well test plate, following the plate format below:

	#1	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**
A	400 µM	200 µM	100 µM	50 µM	25 µM	12.5 µM	6.25 µM	Blank
B	400 µM	200 µM	100 µM	50 µM	25 µM	12.5 µM	6.25 µM	Blank

B. Preparation of test samples

1. Cells or Tissues can be homogenized in 4 volumes of the assay buffer (8588a). Centrifuge the samples at 13,000 ×g for 10 minutes to remove any insoluble material. The soluble fraction may be assayed directly.
2. Samples should be serial diluted to ensure that the readings are within the range of the standard curve. Prepare test samples to a final volume of 20 µL/well in the 96-well flat bottom plate.

C. Working reagent preparation and measurements

1. For each well to be assayed, prepare the working reagent by mixing 62 µL assay buffer (8588a), 16 µL substrate mix (8588c), and 2 µL enzyme mix (8588d).
2. Add 80 µL of the working reagent mix into each well of the 96-well plate containing choline standard, samples, and blank. Incubate the reaction for 60 minutes at room temperature in the dark.
3. Read the absorbance with an ELISA plate reader at 550 nm.

D. Calculations

1. Subtract the OD_{550nm} value of the blank from the OD_{550nm} values of the standards and samples to obtain the ΔOD_{550nm} value.
2. Based on the calibrated ΔOD_{550nm} of the choline standard, make a standard curve by plotting ΔOD_{550nm} as a function of choline concentration (see Figure 1 for a typical standard curve). Determine the equation and R^2 value of the resulting trend line.
3. Suppose the equation of the trend line of the standard curve is $y = Ax + B$, calculate the choline concentration of test samples as follows:

$$[\text{Choline}] = \frac{\Delta OD_{550nm} - B}{A}$$

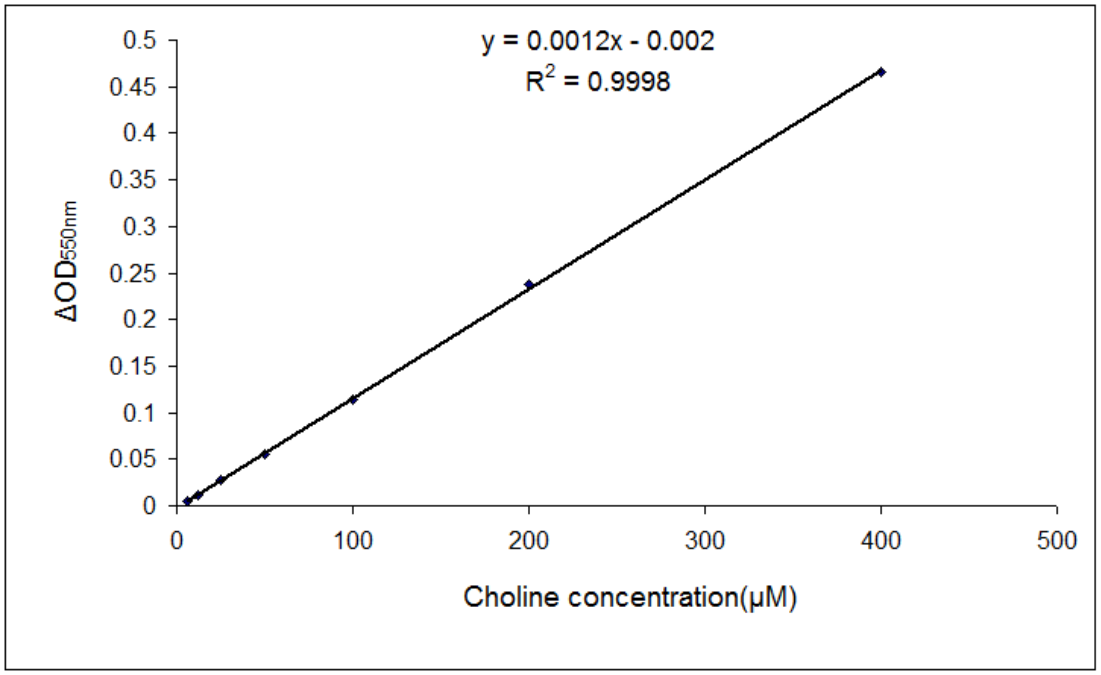

Figure1. A typical choline standard curve measured by ScienCell™ Choline Assay kit.