Phospholipase D Assay (PLD)

Cat. No. 8598
100 Tests in 96-well plate

Introduction

Phospholipase D (PLD) catalyzes the hydrolysis of phosphatidylcholine to generate phosphatidic acid, and subsequently releasing soluble choline. PLD plays an important role in a variety of cellular functions and has been associated with the pathogenesis of diseases such as cancer, Alzheimer's disease, Parkinson's disease, and brain disorders. In this colorimetric assay, phosphatidylcholine is converted to choline by PLD, where choline is then oxidized by choline oxidase and the formed hydrogen peroxide is catalyzed by peroxidase. This product reacts with 4-aminoantipyrine to form a colored solution that can be read at 550nm. The intensity of the colored reaction product is directly proportional to the activity of PLD in the sample.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8598a	1	Assay buffer	25 mL	4°C
8598b	1	PLD positive control	20 µL	-20°C
8598c	1	Substrate	0.4 mL	-20°C
8598d	1	Choline standard	1.0 mL	-20°C
8598e	1	Cofactor mix	1.6 mL	-20°C
8598f	1	Enzyme mix	0.2 mL	-20°C

Product Use

The PLD Assay kit measures PLD activity in various types of samples, including tissue and cell lysate. This product is for research purposes only and is not approved for use in animals, humans, or diagnostic procedures.

Quality Control

Diluted PLD positive control is measured with the PLD Assay kit after various reaction times (Figure 2). Linear detection range is 0.00016 to 0.052 U/ml in 96-well plate assay.

Shipping

Shipped on dry ice.

Positive Control Preparation

1. Diluted PLD positive control: Add 1 µl of PLD positive control into 9 µl of assay buffer (8598a). Prepare diluted PLD positive control to a final volume of 10 µL/well in a 96-well flat bottom plate.

Procedures (96-well plate)

A. Preparation of choline standard

1. Add 60 µL of choline standard (8598d) to 15 µL of assay buffer (8598a) to make a 75 µL solution of 800 µM choline.
2. Obtain 8 test tubes and label them #1 through #8. Add 25 µL of assay buffer (8598a) into tubes #2-7.
3. Add 25 µL of 800 µM choline solution into tube #1.
4. Transfer 25 µL of the 800 µM choline standard into tube #2 and mix well to obtain the 400 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 blank.
6. Obtain a 96-well test plate, prepare 2 replicates (A, B) of each choline standard by aliquoting 10 µL/well of each choline standard into duplicate wells of the 96-well test plate, following plate format below:

	#1	#2	#3	#4	#5	#6	#7	#8
A	800 µM	400 µM	200 µM	100 µM	50 µM	25 µM	12.5 µM	Blank
B	800 µM	400 µM	200 µM	100 µM	50 µM	25 µM	12.5 µM	Blank

B. Preparation of test samples

1. Cells or Tissues can be homogenized in 4 volumes of the assay buffer (8598a). Centrifuge the samples at 13,000 ×g for 10 minutes to remove insoluble material. The soluble fraction may be assayed directly.
2. Samples should be serial diluted to make sure the readings are within the range of the standard curve. Prepare test samples to a final volume of 10 µL/well in the 96-well flat bottom plate.

C. Preparation of working reagents and measurements

1. Prepare the appropriate volume of PLD assay working reagent based on the number of samples to be measured. For each well of choline standard and blank, prepare the working reagent by mixing 72 µL of assay buffer (8598a), 16 µL of cofactor mix (8598e), and 2 µL of enzyme mix (8598f). For each well of sample and diluted PLD positive control, prepare the working reagent by mixing 68 µL of assay buffer (8598a), 16 µL of cofactor mix (8598e), 4 µL of substrate mix (8598c), and 2 µL of enzyme mix (8598f).
2. Add 90 µL of the working reagent mix into each well of the 96-well plate containing choline standard, diluted PLD positive control, sample, and blank. Incubate the reaction for 20 minutes at room temperature in the dark.
3. Measure the absorbance readings at 550 nm with an ELISA plate reader at 0 minutes and 20 minutes.

D. Calculations

1. Subtract the OD_{550nm} value of the blank from the OD_{550nm} values obtained for all the standards and samples to get the ΔOD_{550nm} values.
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 slope and R^2 value of the trend line.

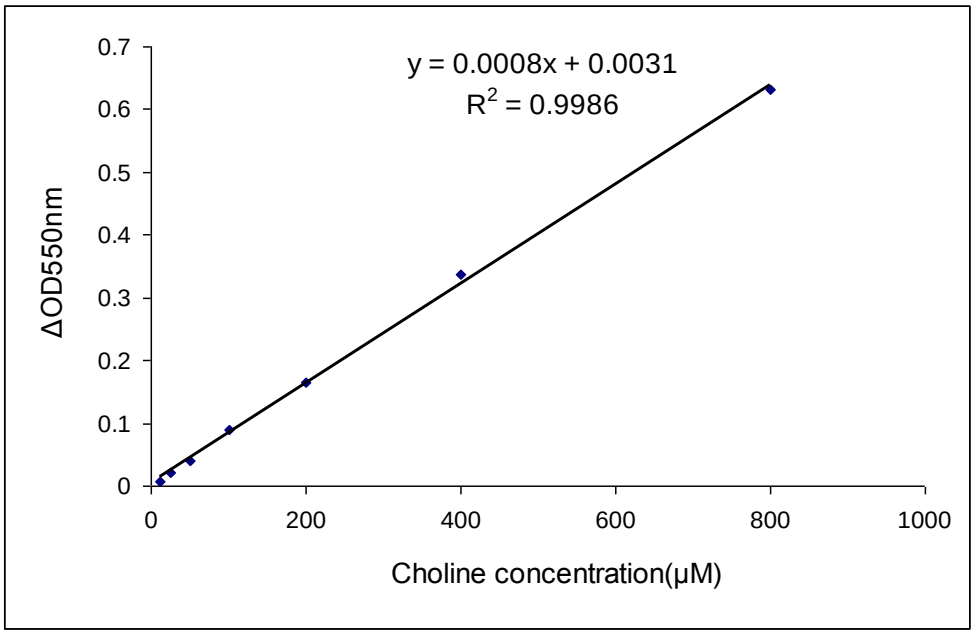

Figure 1. A typical choline standard curve measured by ScienCell™ Phospholipase D Assay kit.

3. Calculate the PLD activity of samples and diluted PLD positive control using the following formula:

$$[\text{Phospholipase D}] = \frac{T_{20} - T_0}{\text{Slope X } 20} \text{ x sample dilution}$$

Note: T_{20} and T_0 are absorbance readings of the sample and positive control at 20 minutes and 0 minutes, respectively. 20 is the enzyme reaction time.

Unit definition: One unit catalyzes the formation of 1.0 µmol of choline per minute at pH 8.0 at 25 ºC.

4. Obtain the change in absorbance T_{20} and T_0 based on the curve of diluted PLD positive control, as shown in Figure 2.

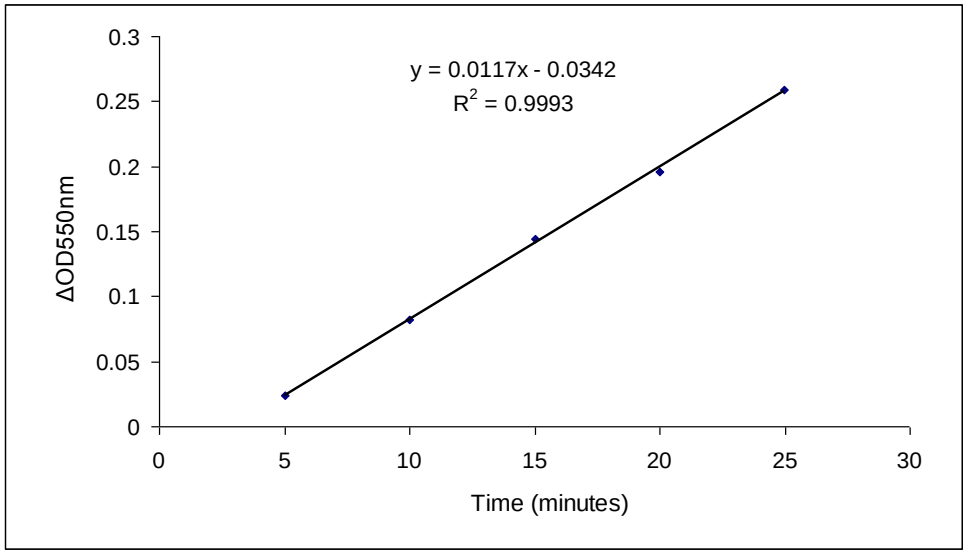

Figure 2. The change in absorbance the ΔOD_{550nm} of diluted PLD positive control during the time at 550nm.

5. Use the formula below to calculate the activity of PLD positive control:

$$[\text{Phospholipase D}] = \frac{0.196}{0.0008 \text{ X } 20} \text{ x } 10 = 12.25 \text{ (nmol/min/ml)} = 0.01225 \text{ (U/ml)}$$