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Product Information

Phospholipase D Assay Kit

Catalog Number **MAK137**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Phospholipase D (PLD) catalyzes the hydrolysis of the phosphodiester bond of glycerophospholipids to generate phosphatidic acid and a free headgroup. Abnormalities in PLD expression have been associated with human cancers.

The Phospholipase D Assay Kit provides a simple and high-throughput adaptable assay for measuring PLD activity in biological samples. In this assay, PLD hydrolyzes phosphatidylcholine to choline, which is determined using choline oxidase resulting in a colorimetric (570nm) /fluorometric(λ_{ex} = 530/ λ_{em} = 585 nm) product, proportional to the PLD activity in the sample.

Unit definition: one unit of PLD catalyzes the formation of 1 μmole choline per minute under the assay conditions (pH 7.4).

Components

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

Assay Buffer Catalog Number MAK137A	10 mL
Enzyme Mix Catalog Number MAK137B	1 vl
Calibrator Catalog Number MAK137C	400 μL
Dye Reagent Catalog Number MAK137D	120 μL
Substrate Catalog Number MAK137E	1.5 mL

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.
- 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

Use ultrapure water for the preparation of reagents. Equilibrate all components to room temperature before use. Briefly centrifuge vials before opening. Keep thawed tubes on ice during assay.

Enzyme Mix – Reconstitute in 120 μL of Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Use within 1 month of reconstitution and keep cold while in use.

Storage/Stability

The kit is shipped on dry ice and storage at –20 °C, protected from light, is recommended.

Procedure

<u>Calibrator for Colorimetric Detection</u>

Add 33 μL of the Calibrator to 187 μL of water to prepare a 300 μM Calibrator (Choline) working solution solution. Add 0, 30, 60, and 100 μL of the 300 μM Calibrator solution into tubes. Add water to each tube to bring the volume up to 100 μL, generating 0 (blank), 90, 180, and 300 μM standards. Transfer 10 μL of calibrators into separate wells of 96 well plate.

Calibrator for Fluorimetric Detection

Further dilute the 300 μM Calibrator solution by adding 20 μL of the 300 μM Calibrator solution to 180 μL of water to prepare a 30 μM Calibrator solution. Add 0, 30, 60, and 100 μL of the 30 μM Calibrator solution into tubes, generating 0 (blank), 9, 18, and 30 μM standards. Add water to each tube to bring the volume to 100 μL. Transfer 10 μL of calibrators into separate wells of 96 well plate.

Sample Preparation

Liquid samples can be assayed directly. Solid samples should be homogenized in a suitable enzyme buffer prior to assay. Aliquot 10 μL of each sample into separate wells of a 96 well plate.

For unknown samples, it is suggested to test several sample dilutions.

Notes: Thiol (SH)-containing reagents (e.g., dithio-threitol, β-mercaptoethanol, >5 μM), sodium azide, EDTA, and sodium dodecyl sulfate are known to interfere with this assay and should be avoided in sample preparation.

If a sample is known to contain choline, it should be removed by dialysis or membrane filtration.

Assay Reaction

1. Set up the Master Reaction Mix according to the scheme in Table 1. 90 μL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Sample and Calibrators
Assay Buffer	85 μL
Enzyme Mix	1 μL
Dye Reagent	1 μL
Substrate	12 μL

2. Add 90 μL of the Master Reaction Mix to each of the sample and calibrator wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction at room temperature or 37 °C. Protect the plate from light during the incubation.

3. After 10 minutes, take the initial measurement. For colorimetric assays, measure the absorbance at 570 nm $(A_{570})_{initial}$. For Flurometric assays, measure the fluorescence intensity ($FLU_{initial}$, λ_{ex} = 530/ λ_{em} = 585 nm).

4. Incubate the plate an additional 20 minutes (30 minutes total) and then measure the samples again to determine the $(A_{570})_{final}$ or $(FLU)_{final}$.

Results

Subtract blank value (0 calibrator) from the calibrator values and plot the absorbance or fluorescence measured for each calibrator against the calibrator concentrations. Determine the slope of the calibrator curve.

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

Use the slope of the calibrator curve to calculate the phospholipase D activity of the sample:

$$\text{PLD Activity (units/L)} = \frac{(A_{570})_{final} - (A_{570})_{initial}}{\text{Slope} \times t} \times n$$

$$\text{PLD Activity (units/L)} = \frac{(FLU)_{final} - (FLU)_{initial}}{\text{Slope} \times t} \times n$$

where:

t = enzyme reaction time (20 minutes in standard assay)

n = dilution factor

Note: If the calculated PLD activity of a sample is higher than 10 units/L in the colorometric assay or 1 units/L in the fluorometric assay, dilute sample in assay buffer and repeat the assay. Multiply the results by the dilution factor.

Unit definition: one unit of PLD catalyzes the formation of 1 μmole choline per minute under the assay conditions (pH 7.4).

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	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 fluorescence assays, use black plates with clear bottoms. 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
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use 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 calibrators	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 calibrator curve	Pipetting errors in preparation of calibrator	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 plate well
	Calibrator stock is at incorrect concentration	Refer to the calibrator 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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