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Phospholipase D Activity Colorimetric Assay Kit

rev. 10/13

(Catalog # K725-100; 100 assays; Store at -20°C)

I. Introduction:

Phospholipase D (PLD) is a key player in phospholipid metabolism. PLD hydrolyzes the phosphodiester bond of the glycerophospholipids, resulting in the production of phosphatidic acid and a free headgroup. PLD activity regulates the actin cytoskeleton, vesicle trafficking for secretion and endocytosis, and receptor signaling. Abnormalities in PLD activity and expression have been associated with Alzheimer's disease, stroke, cancer and other brain disorders. BioVision's Phospholipase D Activity Assay Kit provides a simple and sensitive method of measuring PLD activity using colorimetry (OD 570 nm). In this assay, PLD cleaves choline from Phosphatidylcholine. The free choline is then oxidized by PLD enzyme mix to generate an intermediate which reacts with PLD Probe to generate color (OD 570 nm). This high-throughput adaptable assay kit can detect PLD activity as low as 1.0 mU/ml in a variety of samples.

II. Application:

- Measurement of PLD activity in various tissues/cells
- Analyzing intracellular signaling
- Screening of PLD inhibitors

III. Sample Type:

- Animal tissues: liver, brain, kidney, etc.
- Cell culture: Adherent or suspension cells

IV. Kit Contents:

Components	K725-100	Cap Code	Part Number
PLD Assay Buffer	25 ml	WM	K725-100-1
PLD Probe (in DMSO)	200 µl	Red	K725-100-2A
PLD Substrate	100 µl	Blue	K725-100-3
PLD Enzyme Mix (Lyophilized)	1 vial	Green	K725-100-4
Choline Standard (Lyophilized)	1 vial	Yellow	K725-100-5
PLD Positive Control (Lyophilized)	1 vial	Orange	K725-100-6

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

VI. Storage and Handling:

Store kit at -20°C, protected from light. Read the entire protocol before performing the experiment.

VII. Reagent Preparation and Storage Conditions:

- **PLD Assay Buffer:** Warm Assay Buffer to room temperature before use. Store at -20°C. Use within two months.
- **PLD Probe:** Warm to room temperature before use. Store at -20°C. Use within two months.
- **PLD Substrate:** Ready to use as supplied. Store at -20°C. Use within two months.
- **PLD Enzyme Mix:** Reconstitute with 220 µl PLD Assay Buffer. Aliquot and store at -20°C. Stable for two months.
- **Choline Standard:** Reconstitute with 100 µl PLD Assay Buffer to generate 50 mM Choline Standard Solution. Use within two months.
- **PLD Positive Control:** Reconstitute with 20 µl PLD Assay Buffer. Aliquot and store at -20°C. Use within two months.

VIII. Phospholipase D Assay Protocol:

1. **Sample Preparation:** Homogenize 10 mg of sample (wet weight or cell pellet) in 100 µl PLD Assay Buffer. Centrifuge at 10,000 X g for 5 min. at 4°C. Collect the supernatant. Add 1-5 µl of supernatant into a 96-well plate and adjust the volume to 50 µl with PLD Assay Buffer. Add 2 µl of PLD Positive Control into desired wells(s) and adjust the final volume to 50 µl with PLD Assay Buffer.

   Notes:

   a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

   b. For samples having background, prepare parallel well(s) containing the same amount of sample as in the test well (sample background control). Adjust the volume to 50 µl with PLD Assay Buffer.

   c. 1% or more of Triton X-100 will interfere with the assay.

2. **Standard Curve Preparation:** Dilute Choline Standard to 0.5 mM by adding 10 µl of 50 mM Choline Standard into 990 µl of PLD Assay Buffer, mix well. Add 0, 2, 4, 6, 8 and 10 µl of the diluted 0.5 mM Choline Standard into a series of wells in 96-well plate to generate 0, 1, 2, 3, 4 and 5 nmol/well of Choline Standard. Adjust the volume to 50 µl/well with PLD Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays (samples, Standards & Positive Control) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	*Background Control Mix
PLD Assay Buffer	45 µl	46 µl
PLD Enzyme Mix	2 µl	2 µl
PLD Probe	2 µl	2 µl
PLD Substrate	1 µl	----

** Mix well by quick vortexing. Make sure PLD Substrate is completely dissolved. Add 50 µl of Reaction Mix to each well containing the Choline Standards, Positive Control and samples. Mix well.

Note:

* For samples having high background, add 50 µl of Background Control Mix to sample background control well(s). Mix well.

** We recommend mixing the Reaction Mix immediately after adding all the components.

4. **Measurement:** Incubate for 20-30 min. at 25°C and measure absorbance (OD 570 nm).

   Note: Incubation time depends on the PLD Activity in the samples. We recommend measuring OD in a kinetic mode, and choosing two time points (T_1 and T_2) in the linear range (OD values A_1 and A_2 respectively) to calculate the PLD activity of the samples. The Standard Curve can be read in end point mode (i.e. at the end of incubation time).

5. **Calculations:** Subtract 0 Choline Standard reading from all Standard readings. Plot the Choline Standard Curve. If sample background control reading is significant, subtract background control reading from sample readings. Calculate the PLD activity of the test sample: $\Delta OD = A_2 - A_1$. Apply ΔOD to the Choline Standard Curve to get B nmol of Choline generated by PLD during the reaction time ($\Delta T = T_2 - T_1$).

Sample PLD Activity = B/(ΔT X V) x D = nmol/min/ml = mU/ml

Where: **B** is the Choline amount from the Standard Curve (nmol)
ΔT is the reaction time (min.)
V is the sample volume added into the reaction well (ml)
D is the sample dilution factor

Unit Definition: One unit of PLD activity is the amount of enzyme that will generate 1.0 µmol of Choline per min. at pH 7.4 at 25°C.

(a) (b)

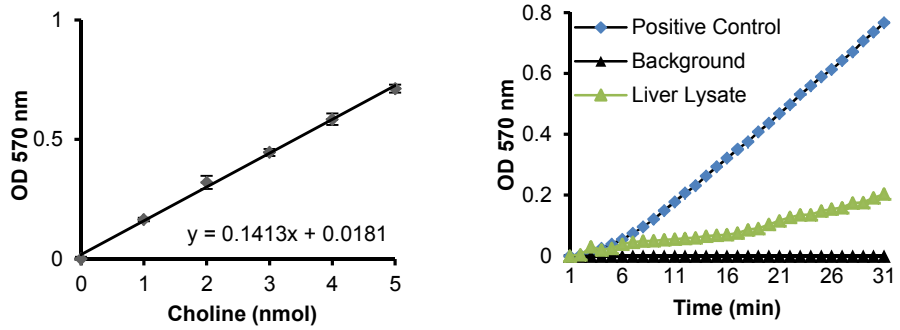

Figure: Choline Standard Curve (a). PLD activity in liver lysate (5 µl) & Positive Control (1 µl) (b). Assays were performed following the kit protocol.

IX RELATED PRODUCTS:

Choline/Acetylcholine Quantification Colorimetric/Fluorometric Kit (K615)
Phosphatidylcholine Colorimetric/Fluorometric Kit (K576)
Sphingomylein Quantification Colorimetric Assay Kit (K600)
Cholesterol/Cholesteryl Ester Quantification Colorimetric Kit II (K623)
Cholesterol/Cholesteryl Ester Quantification Colorimetric/Fluorometric Kit (K603)
Lipase Activity Colorimetric Assay Kit (K722, K723)
Lipase Activity Fluorometric Assay Kit (K724)

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