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Total Phosphodiesterase Activity Assay Kit (Fluorometric)

(Catalog # K927-100; 100 assays; Store at -20°C)

I. Introduction:

Phosphodiesterase (PDE) is a family of enzymes that cleave a phosphodiester bond. These include cyclic nucleotide phosphodiesterases, phospholipase C and D, RNases, DNases and some restriction endonucleases. Phosphodiesterases are important enzymes used for a variety of biological functions including metabolism of second messenger molecules like cAMP / cGMP (therefore, regulation of their intracellular levels). PDE activity is responsible for degradation of mRNA after it had been translated to protein and of DNA during apoptosis. Some PDE isoforms such as PDE 5 and PDE 11 are therapeutic targets because of their role in the cardiovascular system and tumor formation respectively. BioVision's PDE assay kit is a fluorometric plate-based assay for kinetic measurement of total phosphodiesterase activity. It is based on the cleavage of a coumarin based synthetic substrate by phosphodiesterases to generate a product, which fluoresces at Ex/Em= 370/450 nm. It does not rely on the quantification of phosphate released by phosphodiesterase action, like other traditional methods currently in use. Therefore, sample background is negligible, and does not interfere with the assay. The substrate is specific for phosphodiesterases and is not cleaved by enzymes that act on carboxyl esters or by phosphatases. This assay can detect as low as 0.25 µU of total PDE.

PDE Substrate $\xrightarrow{\text{PDE}}$ Fluorescent product (Ex/Em= 370/450 nm)

II. Applications:

- Measurement of Phosphodiesterase activity in various cells/ biological fluids

III. Sample Type:

- Cell lysate
- Biological fluids, (eg. cerebrospinal fluid, CSF)

IV. Kit Contents:

Components	K927-100	Cap Code	Part Number
PDE Assay Buffer	25 ml	WM	K927-100-1
PDE Substrate	15 µl	Red	K927-100-2
Coumarin Standard (50 µM)	100 µl	Yellow	K927-100-3
PDE Positive Control	1 vial	Purple	K927-100-4

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer
- Anhydrous DMSO

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. **Briefly centrifuge small vials prior to opening**. Read entire protocol before performing the assay.

- **PDE Assay Buffer:** Warm to room temperature before use.
- **PDE Substrate:** Store at -20ºC. Reconstitute vial with 135 µl DMSO before use and mix well. Aliquot and store at -20ºC
- **Coumarin Standard:** Store at -20ºC. Thaw completely before using. Mix well by pipetting or vortexing. Centrifuge before opening.
- **PDE Positive Control**: Lyophilized positive control is stable at -20°C for 1 year. Reconstitute with 22 µl PDE Assay buffer before use. Aliquot the remaining positive control and store at -80°C. Use reconstituted positive control within 2 months.

VII. Phosphodiesterase Activity Assay Protocol:

1. **Sample Preparation:** Homogenize cells (1 x 10^6) with 100 µl ice cold PDE Assay Buffer, and keep on ice for 10 min. Centrifuge at 10,000 x g for 10 minutes at 4°C and transfer the supernatant to a fresh tube. Determine protein concentration using preferred method. *We recommend BV Cat. No. K813-2500.* Protein concentration should range between 5-20 mg/ml. Concentrated samples may be diluted with PDE assay buffer. Aliquot and store lysates at -80°C unless being used immediately. Biological fluids samples can be used directly. Use 5-20 µl sample per well. Prepare two identical wells for each sample labelled "Sample Background Control" (SBC), and "Sample" (S) respectively. Adjust volume in each well to 50 µl with PDE Assay Buffer. For positive control (PC), add 4 µl of PDE Positive Control into desired well and adjust the final volume to 50 µl with PDE Assay Buffer. For assay background control (BC), add 50 µl of PDE buffer to a well.

   Note: For unknown samples, we suggest testing several concentrations to ensure the readings are within the Standard Curve range.

2. **Coumarin Standard Curve Generation:** Dilute the provided 50 µM Coumarin standard 1:20 in DMSO to obtain 2.5 µM stock**.**. Mix well. Add 0, 2, 4. 8, 12 and 16 µl form the 250 nM Coumarin stock into a series of wells in a white 96 well plate. Add PDE Assay Buffer to each well and bring up the total volume to 100 µl to generate 0, 5, 10, 20, 30 and 40 pmol / well of Coumarin standard. Mix well by pipetting. *Make sure that no bubbles are introduced in the wells*.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. Add 50 µl SBC Mix to each of the "Sample background control" wells and 50 µl Reaction Mix to wells containing samples (S), positive control (PC) and background control (BC). For each well, prepare 50 µl:

	SBC Mix	Reaction Mix
PDE Assay Buffer	50 µl	48.5 µl
PDE Substrate	-	1.5 µl

The volume at this stage in every well (i.e. SBC, S, BC and PC standards) is 100 µl.

Notes:

a. Turbidity upon addition of PDE Substrate to PDE Assay buffer is normal and will disappear by vortexing or pipetting few times.
b. Have the plate reader ready at Ex/Em 370/450 nm on kinetic mode.

4. **Measurement:** Immediately start recording fluorescence at 30 second intervals for 30 - 60 minutes at RT.

   Note: Incubation time depends on the PDE activity in samples. We recommend measuring the RFU in kinetic mode and choosing two time points (t_1 & t_2) in the linear range to calculate the enzymatic activity of the samples. The Coumarin Standard Curve can be read in endpoint mode.

5. **Calculation:** Subtract the standard background (0 pmol Coumarin) from standard readings, and sample background control (BC) RFU values from the sample (S) RFU values respectively. *If sample background controls (SBC) RFUs are higher than BC, subtract SBC RFUs from sample RFUs instead*. Estimate amount of coumarin in the reaction using the standard curve. Calculate ΔM, which is the change in amount of coumarin between time t_1 and t_2. PDE Activity may be calculated using the following equations:

Activity = ΔM / (Δt x v) x D (pmol / (min x ml) = µUnits / ml)

Where: **ΔM =** change in coumarin concentration during Δt (pmol)
Δt = t2 – t1 (min)
v = volume of biological fluid added to well (ml)
D = dilution factor

or

Activity = ΔM / (Δt x p) x D (pmol / (min x mg) = µUnits / mg)

Where: **ΔM =** change in coumarin concentration during Δt (pmol)
Δt = $t_2 – t_1$ (min)
p = sample protein concentration (mg)
D = dilution factor

Unit Definition: One unit of PDE is the amount of enzyme that generates 1.0 µmol of coumarin per minute at pH 8.8 at RT.

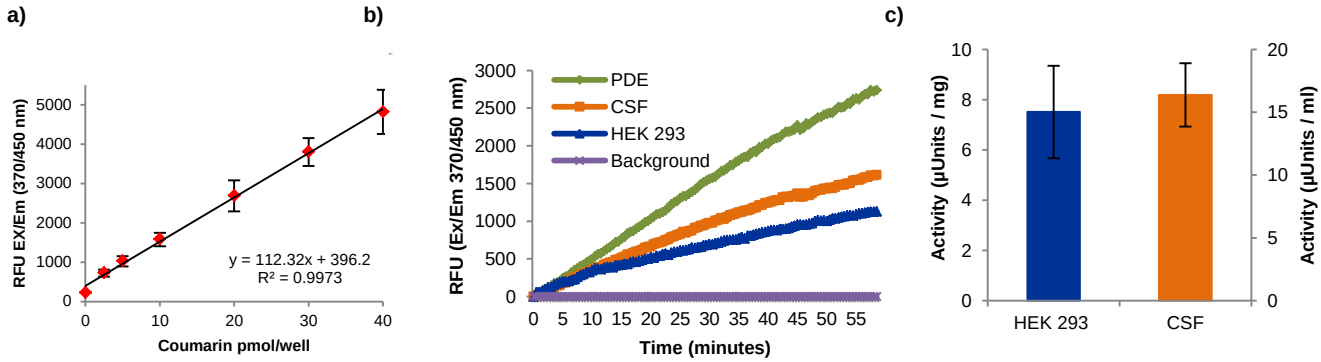

Figure: (a) Coumarin standard curve (b) Phosphodiesterase reaction in PDE (positive control), HEK 293 (40 µg protein) cells and normal human CSF (20 µl undiluted). (c) Phosphodiesterase activity in HEK 293 cells and CSF. Assays were performed following kit protocols.

VIII. RELATED PRODUCTS:

Acetylcholinesterase Activity Colorimetric Assay Kit (K764)
Cholinesterase Activity Assay Kit (Colorimetric) (975)
Alkaline Phosphatase Activity Colorimetric Assay Kit (K412)
BCA Protein Assay Kit (K813)
PDE3B Blocking Peptide (3956BP-50)
IBMX (1714-100)
Cilostazol (2393-25)
Butyrylcholinesterase Activity Kit (Colorimetric) (K516)
Acid Phosphatase Activity Colorimetric Assay Kit (411)
Alkaline Phosphatase Activity Fluorometric Assay Kit (K422)
PDE3B Antibody (3956-200)
Calmodulin, human recombinant (7838-500)
(R,S)-Rolipram (2010-5)
Milrinone (2163-25)

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