

FAM-cAMP PDE IV substrate *Green Fluorescence*

Catalog number: 13602 Unit size: 0.5 umol

Component	Storage	Amount
FAM-cAMP PDE IV substrate *Green Fluorescence*	Freeze (<-15 °C), Minimize light exposure	0.5 umol

OVERVIEW

This green fluorescent cAMP derivative is a specific substrate for phosphodiesterase (PDE) IV. It can be used for assaying PDE IV activities or screening PDE IV inhibitors in combination with anti-CAMP antibody in a FRET readout or FP format. PDE is a group of enzymes that degrade the second messenger molecules: cyclic nucleotides cAMP and cGMP. They regulate the localization, duration, and amplitude of cyclic nucleotide signaling within subcellular domains. PDEs are therefore important regulators of signal transduction mediated by these second messenger molecules. PDE enzymes are often targets for pharmacological inhibition due to their unique tissue distribution, structural and functional properties. Inhibitors of PDE can prolong or enhance the effects of physiological processes mediated by cAMP or cGMP by inhibition of their degradation by PDE. PDE inhibitors have been identified as new potential therapeutics in areas such as pulmonary arterial hypertension, coronary heart disease, dementia, depression and schizophrenia.

AT A GLANCE

Important Following protocol only provides a guideline, and should be modified according to your specific needs.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	515 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 $^\circ$ C after preparation. Avoid repeated freeze-thaw cycles.

1. FAM-cAMP PDE IV stock solution (1 mM):

Make a 1 mM stock solution by adding 500 μL of DMSO into the vial of 0.5 umol FAM-cAMP PDE IV substrate.

PREPARATION OF WORKING SOLUTION

FAM-Cyclic-3', 5'-AMP PDE IV substrate assay solution (2X):

Make 2X FAM-Cyclic-3', 5'-AMP PDE IV substrate assay solution by diluting 1 mM FAM-Cyclic-3', 5'-AMP PDE IV substrate stock solution into your PDE buffer (such as 10 mM Tris-HCl, pH 7.4, 10 mM Mg Cl_2 , 1 mM $MnCl_2$) to make a 200 - 400 nM solution.

Note Make only sufficient quantity needed for the assay.

SAMPLE EXPERIMENTAL PROTOCOL

- Mix equal volume of the PDE IV standards or samples with 2X FAM-Cyclic-3' ,5'-AMP PDE IV substrate assay solution, and incubate at room temperature for at least 1 hour.
- 2. Monitor the fluorescence polarization at Ex/Em = 490/525 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

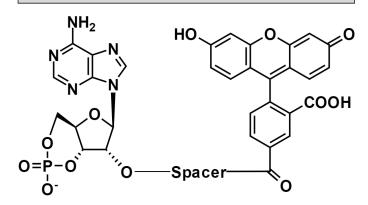


Figure 1. Chemical structure for FAM-cAMP PDE IV substrate *Green Fluorescence*

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