FAM-cGMP PDE V substrate *Green Fluorescence*

Catalog number: 13604
Unit size: 0.5 umol

Component	Storage	Amount
FAM-cGMP PDE V substrate *Green Fluorescence*	Freeze (< -15 °C), Minimize light exposure	1 vial (0.5 umol)

OVERVIEW

This green fluorescent cGMP derivative is a specific substrate for phosphodiesterase (PDE) V. It can be used for assaying PDE V activities or screening PDE V inhibitors in combination with anti-cGMP 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. For example, Sildenafil (Viagra) is an inhibitor of cGMP-specific PDE V, which enhances the vasodilatory effects of cGMP in the corpus cavernosum, and is used to treat erectile dysfunction.

AT A GLANCE

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

KEY PARAMETERS

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 °C after preparation. Avoid repeated freeze-thaw cycles.

FAM-cGMP PDE V stock solution (1 mM)
Make a 1 mM stock solution by adding 500 µL of DMSO into the vial of 0.5 umol FAM-cGMP PDE V substrate.

PREPARATION OF WORKING SOLUTION

FAM-Cyclic-3', 5'-GMP PDE V substrate assay solution (2X)
Make 2X FAM-Cyclic-3', 5'-GMP PDE V substrate assay solution by diluting 1 mM FAM-Cyclic-3' ,5'-GMP PDE V substrate stock solution into your PDE buffer (such as 10 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 1 mM $MnCl_2$) to make a 200 - 400 nM solution.

Note Make only sufficient quantity needed for the assay.

SAMPLE EXPERIMENTAL PROTOCOL

1. Mix equal volume of the PDE V standards or samples with 2X FAM-Cyclic-3' ,5'-GMP PDE V 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

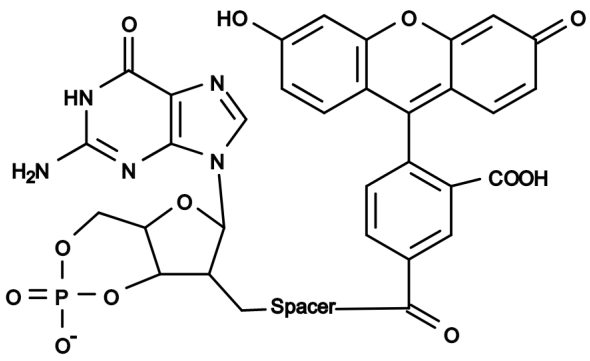

Figure 1. Chemical structure for FAM-cGMP PDE V substrate *Green Fluorescence*

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