

cAMP- or cGMP-specific Phosphodiesterase Activity Kit

Cat. No. KITZ-064 **Lot. No.** (See product label)

SPECIFICATION

Product Overview

Cyclic nucleotide phosphodiesterases (PDEs) are involved in a myriad of cellular processes due to their ability to hydrolyze, and thus control, the levels of the second messenger signaling molecules cAMP and cGMP. PDEs have been implicated in a variety of diseases such as erectile dysfunction, asthma, chronic obstructive pulmonary disease, learning disorders, depression and memory functions, neurodegenerative diseases, autoimmune disorders, heart failure and myocardial infarction. The cAMP- or cGMP-specific Phosphodiesterase Activity Kit is a luminescent, high-throughput screening (HTS) method for measuring cyclic nucleotide phosphodiesterase activity from purified sources. The kit is optimized to work with both cAMP- and cGMP-dependent phosphodiesterases. The total time required for the assay from start to finish is less than 1 hour after the PDE reaction is complete. cAMP- or cGMP-specific Phosphodiesterase Assay is compatible with 96-, 384- and 1536-well plate formats. The assay protocol begins by assembling a PDE reaction using cAMP or cGMP as a substrate at a final concentration of 1 μ M and 10 μ M, respectively. Following completion of the PDE reaction, PDE-Glo Termination Buffer containing the PDE inhibitor IBMX (3-isobutyl-1-methylxanthine, not supplied) is added to inhibit most PDEs; additional inhibitors may need to be added to this buffer. The PDE-Glo Detection Solution is added, then the Kinase-Glo Reagent is added. Luminescence is measured using a plate-reading luminometer. The light output is proportional to PDE activity in the reaction.

Size 1,000 assays

Storage Store the system at –10°C to –30°C.

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Shipping	Ice pack
Kit Components	cAMP, 1mM: 100µl cGMP, 1mM: 500µl PDE-Glo Reaction Buffer, 5X: 1.2ml PDE-Glo Detection Buffer, 5X: 600µl PDE-Glo Termination Buffer, 5X: 600µl Protein Kinase A: 20µl Kinase-Glo Substrate: 1 bottle Kinase-Glo Buffer: 10ml
Materials Required but Not Supplied	1. 100mM 3-isobutyl-1-methylxanthine (IBMX) in 100% DMSO 2. additional PDE inhibitors, if desired 3. white, opaque, polystyrene, nontreated, flat-bottom 96-, 384- or 1536-well plate. Do not use treated plates, black plates or clear plates. 4. luminometer or charge-coupled device (CCD) capable of reading multiwell plates 5. multichannel pipette or automated pipetting station 6. purified active PDE enzyme(s) Note: To assay the PDE type I isoform add Ca²⁺ and calmodulin (CaM) to the PDE-Glo Reaction Buffer prior to assembling the PDE reaction.

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