CREATIVE BIOMART®
Assay Kit

Cyclic Nucleotide Phosphodiesterase assay Kit

Product Information

Cat.No. Kit-0667

Product Overview

Unique, non-radioactive, colorimetric assay.Used to screen inhibitors and modulators of cyclic nucleotide phosphodiesterase activity.HTS format permitting rapid assays of large numbers of samples.

Size

96 wells

Description

A BIOMOL GREEN Quantizyme Assay System. The Cyclic Nucleotide Phosphodiesterase (PDE) Assay Kit combines a special dual enzyme system with the BIOMOL GREEN Reagent for phosphate detection to create a unique, non-radioactive, colorimetric assay. This HTS-friendly, mix and read system may be used to screen inhibitors and modulators of cyclic nucleotide phosphodiesterase activity. 96-well microplate format permits rapid assays of large numbers of samples.The basis for the assay is the cleavage of cAMP or cGMP by a cyclic nucleotide phosphodiesterase. The 5''-nucleotide released is further cleaved into the nucleoside and phosphate by the enzyme 5''-nucleotidase. The phosphate released due to enzymatic cleavage is quantified using BIOMOL GREEN reagent in a modified Malachite Green assay. The kit includes Type I cyclic AMP phosphodiesterase (PDE) for validation purposes.

Applications

Colorimetric detection, HTS

Storage

-80°C

Kit Components

PDE Enzyme (from bovine brain) (5 x 4 U; lyophilized solid, 4 U per vial. 1 U = 1 nmol 3'',5-cAMP to 5''-AMP per minute under the conditions of the linearity assay (30°C, pH 7.4, 200µM 3'',5''-cAMP

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Storage: -70°C, avoid freeze/thaw cycles.5''-Nucleotidase (1ml; 5 kU/µl in 10mM TRIS-hydrochloric acid, pH 7.4, 125mM sodium chloride, 10% glycerol, 1mM magnesium chloride. One U will release 1 pmol phosphate per minute from 200µM 5''-AMP, 30°C in.a reaction buffer of 10mM TRIS-hydrochloric acid, pH 7.4, 0.2mM magnesium chloride). Storage: -70°C3'',5''-cAMP Substrate (2ml; 1mM in assay buffer (10mM TRIS-hydrochloric acid, pH 7.4). Storage: -20 or -70°C3'',5''-cGMP Substrate (2ml; 1mM in assay buffer (10mM TRIS-hydrochloric acid, pH 7.4). Storage: -20 or -70°CPDE Assay Buffer (40ml (2 x 20ml); 10mM TRIS-hydrochloric acid, pH 7.4) Storage: -70°CBiomol Green Reagent (20ml; liquid in screw-cap plastic bottle). Storage: 4°C, long-term at -70°C5''-AMP Standard (1.0ml; 100µM in 10mM TRIS-hydrochloric acid, pH 7.4 buffer). Storage: -20 or -70°C5''-GMP Standard (1.0ml; 100µM in 10mM TRIS-hydrochloric acid, pH 7.4 buffer). Storage: -20 or -70°CInhibitor (IBMX) (200µl; 200µM in assay buffer (10mM TRIS-hydrochloric acid, pH 7.4)). Storage: -20 or -70°CDesalting column and resin (1 column and 1 g of resin). Storage: room temperature, after rehydration store at 4°C½ volume microplate. Storage: Room temperature

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