CREATIVE BIOMART®
Assay Kit

Deubiquitinase Activity Fluorometric Assay Kit

Product Information

Cat.No. Kit-2103

Product Overview

Deubiquitinase Activity Assay Kit provides a straight-forward and general measure of deubiquitinase activity by utilizing a fluorescent deubiquitinase substrate to detect activity as low as 0.25 µU with purified enzyme.

Size

100 assays

Description

Cell activity and viability is tightly regulated by controlling the production and degradation of the thousands of different proteins in the cell. The proteasome is responsible for the majority of cellular protein degradation; however, drugs targeting the proteasome can have side effects caused by the lack of specificity associated with inhibiting the proteasome itself. Altering the ubiquitination state of target proteins is thus appealing as an alternative approach. Modification of the ubiquitin-mediated proteasome pathway has been shown to be a valid mechanism for treating a variety of diseases, all of which involve dysregulation of cellular proteostasis. As such, it is imperative that these ubiquitination signals also be reversible. The enzymes responsible for cleavage, and hence removal of ubiquitin from ubiquitinated proteins, are known as de-ubiquitinating enzymes, or DUBs. They are proteases that hydrolyze the isopeptide bond between an ubiquitin moiety and a lysine residue on its target protein. By removing the ubiquitin molecule, the protein escapes the fate of proteasomal degradation and remains a viable factor in the cell.

Applications

Measurement of DUB activity in various tissues/cell extractsDetermination of DUB activity associated with pathological conditionsCharacterization of activity of purified DUB enzymes

Target Species

Mammalian

Storage

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Store kit at –20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read entire protocol before performing the experiment.DUB Assay Buffer: Ready to use. Warm to room temperature before use. Store at -20°C.1 M DTT: Ready to use. Warm to room temperature before use. Store at -20°C.DUB Substrate and AMC Standard: Ready to use. Warm DUB Substrate and AMC Standard to room temperature before use. Light sensitive. Do not expose components to light for extended periods of time. Store at -20°C. Use within six months.DUB Positive Control: Reconstitute with 22 ul of DUB Assay Buffer with DTT to prepare the stock solution. Aliquot & store at -80°C.Avoid repeated freeze/thaw. Use within two months.

Kit Components

DUB Assay Buffer: 25 ml1 M DTT: 100 µlDUB Substrate (in DMSO): 25 µlDUB Positive Control: 1 vialAMC Standard (1 mM): 100 µlWhite 96-well Half-Area Plate: 1 plate

Detection method Fluorescence (Ex/Em 350/440 nm)

Compatible Sample Types

Purified recombinant proteinCell and tissue lysate

Features & Benefits

• Simple procedure; takes only ~40 min• Fast and convenient

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