

K2031 HDAC Activity Fluorometric Assay Kit

Kit Contents

Components	K2031-100 100 assays	Part Number
Trichostatin A (10 μ M)	1 x 500 μ L	K2031-C-1
HDAC Stop Solution	2 x 1 mL	K2031-C-2
Fluoro-Substrate Peptide (0.2 mM)	1 x 500 μ L	K2031-C-3
Fluoro-Deacetylated Peptide (0.2 mM)	1 x 100 μ L	K2031-C-4
Crude HDAC	1 x 500 μ	K2031-C-5
Developer	1 x 500 μ L	K2031-C-6
HDAC Assay Buffer	2 x 1 mL	K2031-C-7

Introduction

Histone deacetylase (HDAC) is an enzyme that removes acetyl group from a histone and plays an important role in regulating gene expression. Inhibition of HDAC can regulate transcription and induce apoptosis or differentiation in cancer cells. However, screening compounds that inhibit HDAC is difficult due to the lack of convenient way for detecting HDAC activity.

The HDAC Activity Fluorometric Assay Kit provides a fast and convenient way for detection of HDAC activity based on fluorescence method that eliminates radioactivity, chromatography or extractions in traditional assays. The assay needs only two easy steps performed on the same microtiter plate. First, the HDAC substrate that contains an acetylated lysine side chain is incubated with the sample containing HDAC activity (e.g., HeLa nuclear extract). Second, Deacetylation of the HDAC substrate sensitizes the substrate, so that further treatment with the Lysine Developer generates a fluorophore.

The fluorophore can be easily detected using a fluorometer or a fluorescence plate reader. The assay is well suitable for high throughput screening applications.

Key facts

Detection method

Fluorescent

Sample types

Cell culture extracts, Tissue Extracts

Assay type

Enzyme activity

Reactive species

Mammals

Assay time

1h

Assay Platform

Microplate reader

Storage

Shipped at conditions

Dry Ice

Appropriate short-term storage conditions

-80°C

Appropriate long-term storage conditions

Multi

Storage information

Please refer to protocols

Notes

The HDAC Activity Fluorometric Assay Kit detects HDAC activity in lysates.

The HDAC Activity Assay Kit is primarily designed for the evaluation of HDAC inhibitors using a crude HDAC fraction. Also, any cultured primary cell, cell line, or tissue homogenate can be assayed for HDAC activity with the kit if the appropriate dose of HDAC specific inhibitor e.g. Trichostatin A is used.

The HDAC activity assay protocol is based on an acetylated peptide which is conjugated to AMC. AMC is a fluorescent dye and its fluorescence is quenched when conjugated to the peptide. When the HDAC de-acetylates the peptide, it becomes susceptible to cleavage by an enzyme (the Developer component). This then releases free AMC, which can be measured using a fluorescence microplate reader (Ex/Em 355/460 nm).

The HDAC assay has been shown to detect the activity of the HDAC family, at least class I HDACs in Human or animal cell lysates or in column fractions. The assay shows good linearity of sample response. The assay may be used to follow the purification of HDACs or may be used to detect the presence of HDACs in cell lysates.

Applications for this kit include:

1. Monitoring the purification of HDACs including HDAC1, 2, 3 and 8.
2. Screening inhibitors or activators of HDACs.
3. Detecting the effects of pharmacological agents on HDACs.

Background information on HDACs

Histone Deacetylases (HDACs) are a class of enzymes responsible for the deacetylation of lysine residues on the Nterminal part of the core histones (H2A, H2B, H3 and H4), allowing the histones to wrap the DNA more tightly.

HDAC proteins occur in four groups (class I, class IIA, class IIB, class III, class IV) based on function and DNA sequence similarity.

Classes I, IIA and IIB are considered "classical" HDACs whose activities are inhibited by trichostatin A (TSA), whereas class III is a family of NAD⁺-dependent proteins (sirtuins) not affected by TSA. Class IV is considered an atypical class on its own, based solely on DNA sequence similarity to the others. For research use only! Not to be used in humans.

Caution

FOR RESEARCH PURPOSES ONLY.

NOT FOR HUMAN, VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

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