Fluorogenic HDAC Assay Kit

GenWay ID : GWB-4C8F93

Legacy ID: 40-054-165065

DESCRIPTION: The *Fluorogenic HDAC Assay Kit* is a complete assay system designed to measure histone deacetylase (HDAC) class 1 activity in cell extracts, nuclear extracts, immunoprecipitates, and purified enzymes. It comes in a convenient 96-well format, with all the reagents necessary for 100 fluorescent HDAC activity measurements. In addition, the kit includes purified HDAC2 enzyme and a potent HDAC inhibitor, Trichostatin A, for use as a positive and negative control. The *Fluorogenic HDAC Assay Kit* is based on a unique fluorogenic substrate and developer combination. This assay method eliminates dealing with the radioactivity, extraction, and chromatography aspects of traditional assays. Using this kit, only two simple steps on a microtiter plate are needed to analyze the HDAC activity level. First, the HDAC fluorometric substrate, containing an acetylated lysine side chain, is incubated with a sample containing HDAC activity (e.g. HeLa nuclear extract or purified HDAC enzyme). The deacetylation sensitizes the substrate so subsequent treatment with the Lysine Developer produces a fluorophore that can then be measured using a fluorescence reader.

COMPONENTS:

	Reagent	Amount	Storage
Avoid freeze/ thaw cycles!	HDAC2 human recombinant enzyme	2 µg	-80°C
	Fluorogenic HDAC substrate (5 mM)	50 µl	-80°C
	2x HDAC Developer (contains Trichostatin A)	6 ml	-80°C
	Trichostatin A (200 µM)	100 µl	-20°C
	HDAC assay buffer	10 ml	-20°C
	black, low binding NUNC black microtiter plate	1 plate	Room temp.

APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

REFERENCES:

1. A. Ito *et al.* (2001) *EMBO J.* **20** 1331.
2. N.A. Barlev *et al.* (2001) *Mol. Cell* **8** 1243.
3. A. Ito *et al.* (2002) *EMBO J.* **21** 6236.

GenWay Biotech, Inc.
Protein and Antibody Solutions
6777 Nancy Ridge Drive
San Diego, CA 92121
Phone: 858-458-0866
Fax: 858-458-0833
sales@genwaybio.com
www.genwaybio.com

ASSAY PROTOCOL:

Immediately prior to assay:

1) Dilute HDAC substrate 5 mM stock 25-fold with HDAC assay buffer to make a 200 µM solution. (Make only sufficient quantity needed for the assay; store remaining 5 mM stock solution in aliquots at -80°C.)

2) Dilute HDAC2 in HDAC assay buffer to 4 ng/µl (20 ng/reaction)*. Aliquot any remaining enzyme and store undiluted at -80°C. Keep diluted enzyme on ice. Discard any remaining diluted enzyme after use. **Note: optimal enzyme concentration may vary with the specific activity of the enzyme.*

Step 1:
In duplicate, add the reaction mixtures (below) to the microtiter black plate. Incubate at 37 ºC for 30 min.

	Enzyme Positive Control	**Inhibitor Negative Control**	**Test Inhibitor**	**"Blank" Negative Control**
HDAC2 (4 ng/µl)	5 µl	5 µl	5 µl	–
HDAC substrate (200 µM)	5 µl	5 µl	5 µl	5 µl
BSA (1 mg/ml)	5 µl	5 µl	5 µl	5 µl
Trichostatin A	–	5 µl	–	–
Test Inhibitor	–	–	X µl	–
HDAC assay buffer	35 µl	30 µl	35 - X µl	40 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Step2:
Add 50 µl of HDAC assay developer (2x) to each well. Incubate the plate at room temperature for 15 minutes.

Step 3:
Read sample in a microtiter-plate reading fluorimeter capable of excitation at a wavelength in the range of 350-380 nm and detection of emitted light in the range of 440-460 nm. "Blank" value is subtracted from all other values.

GenWay Biotech, Inc.
Protein and Antibody Solutions
6777 Nancy Ridge Drive
San Diego, CA 92121
Phone: 858-458-0866
Fax: 858-458-0833
sales@genwaybio.com
www.genwaybio.com