Colorimetric Histone Deacetylase Activity Assay (HDAC)

Catalog #8888

100 Tests in 96-well plate

Introduction

Histone Deacetylases (HDACs) catalyze the removal of an acetyl group on histone proteins and regulate gene transcription and expression by altering chromatin structure. HDAC activity is important in controlling cell cycle progression and differentiation processes, and excess HDAC activity is potentially associated with malignant transformation. Screening of HDAC inhibitors therefore has profound implications in developing antineoplastic reagents. ScienCell's Colorimetric Histone Deacetylase Activity Assay Kit (HDAC) offers a rapid and sensitive way to determine the HDAC activity in mammalian cells and tissue samples, and to screen potential inhibitor compounds. Briefly, deacetylation of the lysine residue on the substrate by HDACs generates a product which, when incubated with a developer, releases a chromophore that can be measured at an absorbance of 405 nm.

Note: Due to the intrinsic limitation of the detection method, this kit is only suitable for measuring HDAC activity of class I, IIb and IV HDACs, which include HDAC1, HDAC2, HDAC3, HDAC6, HDAC8, HDAC10, and HDAC11.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8888a	1	HDAC assay buffer (5X)	4 mL	-20°C
8888b	1	HDAC substrate	500 µL	-80°C
8888c	1	Deacetylated standard (5 mM)	250 µL	-80°C
8888d	1	Developer solution	1 mL	-80°C
8888e	1	HDAC inhibitor	10 µL	-20°C
8888f	1	HDAC positive control (cell lysate)	25 µL	-80°C

Additional Materials Required (Materials Not Included in Kit)

96-well flat bottom plate

Plate reader or spectrophotometer

Product Use

HDAC is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

The product is shipped on dry ice. Upon receipt, components #8888a and 8888e should be stored at -20°C, #8888b, 8888c, 8888d and 8888f should be stored at -80°C. Protect from light. Repeated freeze/thaw cycles should be avoided. Aliquot if necessary. If stored properly, the kit is good for up to 12 months.

Quality Control

Serially diluted HDAC positive samples are measured using this kit.

Procedure (for a 96-well plate)

Note: Procedure A provides a detailed instruction for obtaining HDAC activity using OD_{405nm}/µg. Please refer to Procedure B for plotting a standard curve using the Deacetylated standard (Cat #8888c) and calculations.

A. Working reagent preparation and measurements

1. In each reaction well, dilute test samples (10-200 µg of nuclear extract or cell lysate per well) to a final volume of 75 µL with ddH_2O. For the negative control sample use 75 µL of ddH_2O.
2. ***(Optional)*** For the positive control, take 5 µL of the HDAC positive control (Cat #8888f), and add 70 µL of ddH_2O. For the inhibition control, dilute a test sample or the positive control sample to a final volume of 73 µL with ddH_2O, then add 2 µL of the HDAC inhibitor (Cat #8888e).

   Note: Positive and inhibition controls are optional. This kit provides sufficient reagents for 5 positive controls and 5 inhibition controls.
3. Add 20 µL of the HDAC assay buffer (5X, Cat #8888a) and 5 µL of the HDAC substrate (Cat #8888b) to each test sample reaction well and mix thoroughly by pipetting.

   Note: Refreeze unused HDAC substrate (Cat #8888b) to -80°C immediately after use. Repeated freeze/thaw cycles should be avoided. Aliquot if necessary.
4. Incubate at 37°C for at least 1 hour. A longer incubation period is recommended (up to 4 hours).
5. Stop the reactions by adding 5 µl of Developer solution (Cat #8888d) to each reaction well and mix thoroughly by pipetting. Incubate the plate at 37°C for 30 minutes.
6. Read samples absorbance at 405 nm on a plate reader or a spectrophotometer. Calculate sample HDAC activity.

$$\Delta OD_{405nm,\ sample} = OD_{405nm,\ sample} - OD_{405nm,\ negative\ control}$$

$$\text{Sample HDAC activity } (OD_{405nm}/\mu g) = \Delta OD_{405nm,\ sample} / \text{Sample mass } (\mu g)$$

B. *(Optional)* Standard curve plotting using the deacetylated standard

Note: Plotting a standard curve using deacetylated standard is optional and should be performed at the same time as Procedure A. This kit provides sufficient reagents for plotting 10 standard curves. When plotting a standard curve, the samples should be prepared at the same time as the test samples described in Procedure A, step 1.

1. In wells #1-7, add the Deacetylated standard (Cat #8888c) and ddH_2O as shown in the table below.

Well #	1	2	3	4	5	6	7
Deacetylated standard (µL)	0	1	2	3	4	5	6
ddH_2O (µL)	10	9	8	7	6	5	4
Amount in well (nmoles)	0	5	10	15	20	25	30

   Note: Refreeze unused Deacetylated standard (Cat #8888c) to -80°C immediately after use. Repeated freeze/thaw cycles should be avoided. Aliquot if necessary.
2. Add 20 µL of the HDAC assay buffer (5X, Cat #8888a) and 70 µL of ddH_2O to 7 wells #1-7 and mix thoroughly by pipetting. Incubate at 37°C together with test samples described in Procedure A, step 4.

3. At the same time as step 5 in Procedure A, add 5 µl of Developer solution (Cat #8888d) to each reaction well and mix thoroughly by pipetting. Incubate the plate at 37°C for 30 minutes.
4. Read samples absorbance at 405 nm on a plate reader or a spectrophotometer. Calculate $\Delta OD_{405\ nm,\ \#n}$ of each deacetylated standard sample.

$$\Delta OD_{405\ nm,\ \#n} = OD_{405\ nm,\ well\ \#n} - OD_{405\ nm,\ well\ \#1}$$

5. Create the standard curve by plotting $\Delta OD_{405\ nm}$ versus the deacetylated standard amount and derive the resulting standard curve equation: y=ax, where ***y*** is the $\Delta OD_{405\ nm}$ value, ***x*** is the deacetylated standard amount, and ***a*** is the standard curve slope.
6. Using the equations below, determine the deacetylated product amount (nmoles) in each test sample reaction well and HDAC activity (pmoles/min/mg) of the sample.

$$\Delta OD_{405nm,\ sample} = OD_{405nm,\ sample} - OD_{405nm,\ negative\ control}$$

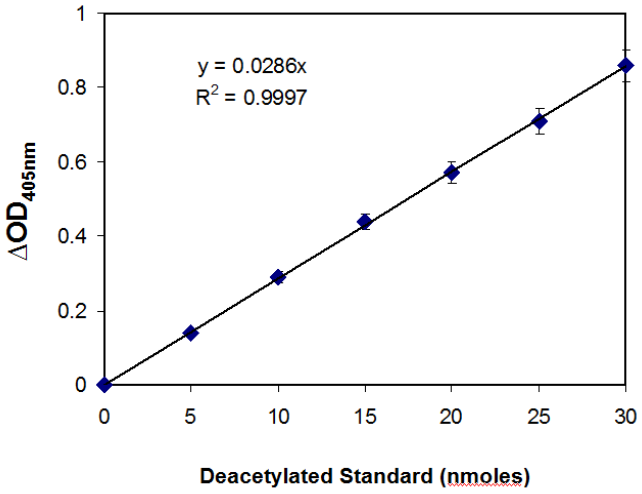
Deacetylated product amount of test sample (nmoles) = $\Delta OD_{405nm,\ sample}$ / ***a***

$$\text{HDAC Activity (pmoles/min/mg)} = \frac{\text{Deacetylated product amount (nmoles) X 1000}}{\text{Incubation time in Procedure A, step 4 (min) X lysate amount in well (mg)}}$$

C. Example calculations

Note: This example shows how to calculate HDAC activity expressed in pmoles/min/mg following the plotting of a standard curve using the deacetylated standard. For calculation of HDAC activity expressed in OD_{405nm}/µg, please refer to Procedure A.

0.05 mg sample was added to the reaction well. Incubation time in Procedure A, step 4 is 150 min. The $\Delta OD_{405\ nm}$ for the test sample is 0.142. The standard curve of the deacetylated standard is shown below:



Deacetylated product amount of test sample = (0.142/0.0286) nmoles = 4.97 nmoles

HDAC activity of test sample = (4.97 nmoles x 1000) / (150 min x 0.05mg)
= 663 pmoles/min/mg