



Histone Deacetylase Fluorometric Assay Kit

Manufactured by BioVision.

ALX-850-290-KI01

~100 tests

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NORTH AMERICA

AXXORA, LLC
6181 Cornerstone Court East
Suite 103
San Diego, CA 92121-4727
Phone: (858) 550-8828
Fax: (858) 550-8825
E-mail: axxora-usa@axxora.com

SWITZERLAND/REST OF THE WORLD

ALEXIS CORPORATION
Industriestrasse 17, Postfach
CH-4415 Lausen / Switzerland
Phone: +41 61 926 89 89
Fax: +41 61 926 89 79
E-mail: alexis-ch@alexis-corp.com

GERMANY

AXXORA DEUTSCHLAND GmbH
Marie-Curie-Strasse 8
DE-79539 Lörrach
Phone: (07621) 5500 522
Fax: (07621) 5500 523
E-mail: axxora-de@axxora.com

UK & IRELAND

AXXORA (UK) LTD.
P.O. Box 6757
Bingham, Nottingham NG13 8LS
Phone: +44 1949 836111
Fax: +44 1949 836222
E-mail: axxora-uk@axxora.com

For laboratory use only. Not for human or diagnostic use.

I. Introduction:

Inhibition of histone deacetylase (HDAC) has been implicated to modulate transcription and to induce apoptosis or differentiation in cancer cells. However, screening of compounds for HDAC inhibition has been difficult due to the lack of convenient tools for analyzing HDAC activity. The new Fluorometric HDAC Activity Assay Kit provides a fast and fluorescence-based method that eliminates radioactivity, extractions, or chromatography, as used in traditional assays. The new procedure requires only two easy steps, both performed in the same microtiter plate. First, the HDAC fluorometric substrate, which comprises an acetylated lysine side chain, is incubated with a sample containing HDAC activity (e.g., HeLa nuclear extract or others). Deacetylation of the substrate sensitizes the substrate, so that, in the second step, treatment with the Lysine Developer produces a fluorophore. The fluorophore can be easily analyzed using a fluorescence plate reader or a fluorometer (Ex(max): 380nm; Em(max): 460nm). The assay is well suited for high throughput screening applications. HDAC inhibitors, substrates, and antibodies are also available separately.

II. Kit Contents:

Components	850-290-KI01	Color Code
	100 tests	Cap Color
HDAC Substrate [Boc-Lys(Ac)-AMC, 4mM]	500 μ l	Amber
10x HDAC Assay Buffer	1 ml	Green
Lysine Developer	1 ml	Orange
HDAC Inhibitor (Trichostatin A, 1mM)	10 μ l	Blue
HeLa Nuclear Extract (5 mg/ml)	10 μ l	Red
Deacetylated Standard [Boc-Lys-AMC, 4mM]	20 μ l	Yellow

III. Histone Deacetylase Assay Protocol:

A. General Considerations:

- Read the entire protocol before beginning the procedure.
- The HeLa extract should be refrozen immediately at -80°C after each use to avoid loss of activity.
- After opening the kit, aliquot the Lysine Developer and refreeze at -20°C for future use.
- If positive and negative controls are designed, the kit provides sufficient reagents for 5 positive control assays with the HeLa Nuclear Extract and 5 Negative Control assays with the HDAC Inhibitor, Trichostatin A.

B. Assay Protocol:

1. Dilute test samples (10-50 μ g of nuclear extract or cell lysate) to 85 μ l (final volume) of ddH₂O in each well (For background reading, add 85 μ l ddH₂O only). For positive control, dilute 2 μ l of HeLa nuclear extract with 83 μ l ddH₂O. For negative control, dilute the sample into 83 μ l of ddH₂O and then add 2 μ l of Trichostatin, or use a known sample containing no HDAC activity.

2. Add 10 μl of the 10X HDAC Assay Buffer to each well.
3. Add 5 μl of the HDAC Fluorometric Substrate to each well. Mix thoroughly.
4. Incubate plates at 37°C for 30 minutes (or longer if desired).
5. Stop the reaction by adding 10 μl of Lysine Developer and mix well. Incubate the plate at 37°C for 30 minutes.
6. Read sample in a fluorescence plate reader with Ex. = 350-380 nm and Em. = 440-460 nm. Signal should be stable for several hours at room temperature. Histone Deacetylase activity can be expressed as the Relative Fluorescence Units per μg protein sample.

C. Standard curve (optional):

1. If desired, a standard curve can be prepared using the known amount of the Deacetylated Standard included in the kit. The exact concentration range of the Deacetylase Standard will vary depending on the fluorometer model, the gate setting, and the exact wavelength used. We recommend starting with a dilution range of 1-20 μM in Assay Buffer.
2. Add 90 μl each of the dilutions and also 10 μl of the 10 x Assay Buffer into a set of wells on the microtiter plate. Use 90 μl of H_2O and 10 μl of 10 x Assay Buffer as zero.
3. Add 10 μl of Lysine Developer to each well and incubate at 37°C for 30 minutes (**Note:** Incubation time should be kept the same for both standard and test samples.)
4. Read samples in a fluorescence plate reader or a fluorometer with Ex. = 350-380 nm and Em. = 440-460 nm.
5. Plot fluorescence signal (y-axis) versus concentration of the Deacetylated Standard (x-axis). Determine the slope as AFU/ μM .
6. Based on the slope, you can determine the absolute amount of deacetylated lysine generated in your sample.

IV. Storage:

Store kit at -80°C . Avoid freeze/thaw cycles.

V. References:

Histone acetylation in chromatin structure and transcription: M. Grunstein; Nature **389**, 349 (1997)

The language of covalent histone modifications: B.D. Strahl and C.D. Allis; Nature **403**, 41 (2000)

Acetylation and chromosomal functions: W.L. Cheung, et al.; Curr. Opin. Cell Biol. **12**, 326 (2000)