ab1438 – HDAC Activity Assay Kit (Fluorometric)

For the high throughput screening of histone deacetylase (HDACs) inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab1438

Storage and Stability

On receipt entire assay kit should be stored at -80°C

Materials Supplied

Item	Quantity	Storage Condition
HDAC Substrate	500 µl	-80°C
10X HDAC Assay Buffer	1.0 mL	-80°C
Lysine Developer	1.0 mL	-80°C
HDAC Inhibitor (Trichostatin A, 1 mM)	10 µl	-80°C
HeLa Nuclear Extract (5 mg/mL)	50 µl	-80°C
Deacetylated Standard (10 mM)	20 µl	-80°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well clear plate with U-shape bottom.
- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Clean Eppendorf tubes for preparing standards or sample dilutions
- Distilled or deionized water
- Precision pipettes with disposable tips

General Notes

- Read the entire protocol before performing the assay.
- The HeLa nuclear extract refrozen immediately -70°C after each use to avoid loss of activity.
- After opening the kit, Lysine Developer should be aliquoted refrozen 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.

Assay Protocol

1. Dilute test samples (10 - 50 µg of nuclear extract or cell lysate) to 85 µl (final volume) of ddH2O in each well (For background reading, add 85 µl ddH2O only). For positive control, dilute 2 µl of HeLa nuclear extract with 83 µl ddH2O. For negative control, dilute the sample into 83 µl of ddH2Oand then add 2 µl of Trichostatin A, 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 min (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 min.
6. Read sample in a fluorescence plate reader (Ex/Em = 350 - 380/440 - 460 nm). The 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.

Calculation (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 10X Assay Buffer into a set of wells on the microtiter plate. Use 90 µl of H2O and 10 µl of 10X Assay Buffer as zero.
3. Add 10 µl of Lysine Developer to each well and incubate at 37◦C for 30 min (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 (Ex/Em = 350 - 380/440 - 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.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)