ab1432 – HDAC Activity Assay Kit (Colorimetric)

For the high throughput screening of histone deacetylase (HDACs) inhibitors.
For research use only - not intended for diagnostic use.
PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab1432

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

Materials Supplied

Item	Quantity	Storage Condition
HDAC Substrate I/HDAC Substrate	500 µl	-80°C
10X Assay Buffer XXX/10X HDAC Assay Buffer	1.0 mL	-80°C
Developer II/Lysine Developer	1.0 mL	-80°C
HDAC Inhibitor/HDAC Inhibitor (Trichostatin A, 1 mM)	10 µl	-80°C
HeLa Nuclear Extract/HeLa Nuclear Extract (5 mg/mL)	50 µl	-80°C
Deacetylated Standard/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 and Developer II/Lysine Developer should be refrozen immediately at –20°C or -70°C after each use to avoid loss of activity.
- 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.
- Using 96-well plates with U-shape bottom. Flat bottom may give a low value.
- Assay Buffer must be at room temperature

Assay Protocol
1. Dilute test samples (50-200 µg of nuclear extract or cell lysate) to 85 µl (final volume) of ddH20 in each well (For background reading, add 85 µl ddH20 only). For positive control, dilute 10 µl of HeLa nuclear extract with 75 µl ddH20. For negative control, dilute your sample into 83 µl of ddH20 and then add 2 µl of Trichostatin, or use a known sample containing no HDAC activity.
2. Add 10 µl of the 10X Assay Buffer XXX/10X HDAC Assay Buffer to each well.
3. Add 5 µl of the HDAC colorimetric substrate to each well. Mix thoroughly.
4. Incubate plates at 37°C for 1 hour (or longer if desired).
5. Stop the reaction by adding 10 µl of Developer II/Lysine Developer and mix well. Incubate the plate at 37°C for 30 min.
6. Read sample in an ELISA plate reader at 400 or 405 nm. Signal is stable for several hours at room temperature. HDAC activity can be expressed as the relative O.D. value per µg protein sample.

Calculation:
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 each individual plate reader and the exact wavelength used. We recommend starting with a dilution range of 10-100 µM in Assay Buffer.
2. Add 90 µl each of the dilutions and also 10 µl of the 10X Assay Buffer XXX/10X Assay Buffer into a set of wells on the microtiter plate. Use 90 µl of H2O and 10 µl of 10X Assay Buffer XXX/10X Assay Buffer as zero
3. Add 10 µl of Developer II/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 an ELISA plate reader at 400 or 405 nm.
5. Plot O.D. value (y-axis) versus concentration of the Deacetylated Standard (x-axis). Determine the slope as ΔO.D./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)