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.

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

Storage and Stability

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

Materials Supplied

All components in this kit are shipped on blue ice and are suitable for storage at -80°C, unless reconstituted. Upon receipt, immediately store kit at -80°C in the dark. Individual components may be stored at alternative temperatures as show in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Item	Quantity	Storage Condition
HDAC Substrate I	500 µl	-80°C
10X Assay Buffer 30	1 mL	-20°C
Developer Mix M	1 mL	-80°C
HDAC Inhibitor	10 µl	-80°C
HeLa Nuclear Extract	50 µl	-80°C
Deacetylated Standard (10mM)	20 µl	-80°C

PLEASE NOTE: 10X Assay Buffer 30 was previously labelled as 10X Assay Buffer XXX and 10X HDAC Assay Buffer, and Developer Mix M as Developer II and Lysine Developer. The composition has not changed.

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 M 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.
- 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 30 to each well.
3. Add 5 µl of the HDAC Substrate I 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 M 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 (10mM) included in the kit. The exact concentration range of the Deacetylase Standard (10mM) 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 30 into a set of wells on the microtiter plate. Use 90 µl of H2O and 10 µl of 10X Assay Buffer 30 as zero
3. Add 10 µl of Developer M 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

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