
Certificate of Analysis

HDAC Assay Kit (Colorimetric Detection)

Catalog # 17-374

Lot # 33640

Kit Components

Half volume clear plate

One half volume 96 well clear plate.

HDAC Assay Buffer, 10X

Catalog # 20-311, Lot # 30422.

One vial containing 1ml of 250mM Tris pH 8.0,
1.37M NaCl, 27mM KCl, 10mM MgCl₂.

Activator Solution

Catalog # 20-268, Lot # 32304.

One vial containing 200µl of activator solution, **20X stock**. Aliquot upon receipt as needed to avoid future freeze-thaw cycles.

HDAC Assay Substrate, Colorimetric Detection

Catalog # 12-561, Lot # 33639.

One vial containing 100µl of 40mM substrate in DMSO.

HDAC Assay Standard, Colorimetric Detection

Catalog # 12-562, Lot # 33638.

One vial containing 50µl of 20mM unacetylated standard in DMSO.

Trichostatin A

Catalog # 20-269, Lot # 31967.

One vial containing 100µl of 200µM Trichostatin A in DMSO.

HeLa Nuclear Extract

Catalog # 12-309, Lot # 32365.

Two vials, each vial containing 50µg in 25µl of nuclear extract prepared from human HeLa cells in RIPA buffer. Aliquot upon receipt as needed to avoid future freeze-thaw cycles.

**FOR RESEARCH USE ONLY
NOT RECOMMENDED OR INTENDED FOR DIAGNOSIS OF DISEASE IN HUMANS
DO NOT USE IN HUMANS**

Kit Description

Quantity: Sufficient reagents for 96 assays per kit.

Storage: Upon receipt, see individual components for storage conditions. The half volume 96 well plate should be stored at room temperature. HeLa Nuclear Extract (Catalog # 12-309) may be stored at -20°C or -70°C. All other components are stored at -20°C.

Stability: Components are stable for 6 months from date of shipment if stored and handled correctly.

Use: This assay is a simple two-step procedure performed in a microtiter plate. In the first step, samples are incubated with the HDAC assay substrate, allowing deacetylation of the substrate. Next, the Activator Solution releases p-nitroanilide from the deacetylated substrate or standard.

Please refer to the User Manual for further information and a detailed assay procedure.

Quality Control Testing

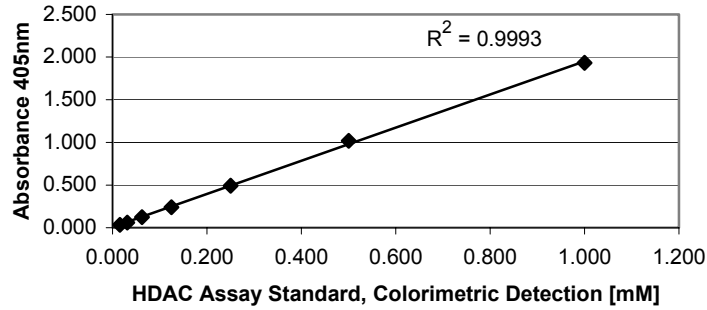


Figure 1. HDAC Assay Standard Curve. Representative data from a previous lot. Two fold serial dilutions of the HDAC Assay Standard were incubated for 20 minutes at room temperature with HDAC Activator Solution as described in the protocol. Points are means of duplicates.

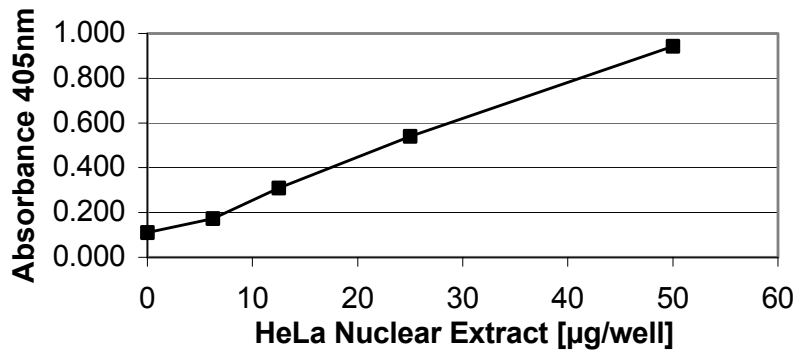


Figure 2. Deacetylation of HDAC Assay Substrate by HeLa Nuclear Extract. Representative data from a previous lot. Indicated concentrations of HeLa Nuclear Extract were incubated at 37°C with 1mM HDAC Assay Substrate for 60 minutes prior to the addition of HDAC Activator Solution as described in the protocol. Points are means of duplicates.

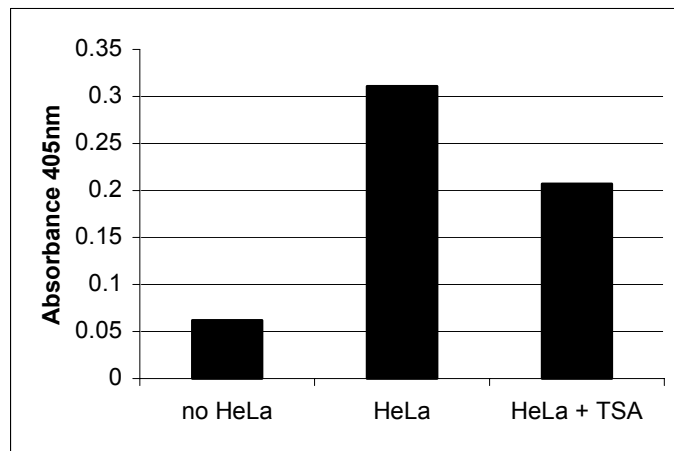


Figure 3. Deacetylation of HDAC Assay Substrate by HeLa Nuclear Extract and Inhibition by Trichostatin A. Representative data from a previous lot. HeLa Nuclear Extract (20µg) was incubated at room temperature with 1mM HDAC Assay Substrate in the absence or presence of 1µM Trichostatin A. Reactions were terminated after 60 minutes with HDAC Activator Solution and absorbance read at 405nm. Points are means of duplicates.