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## Certificate of Analysis

### HDAC Assay Kit (Colorimetric Detection)

Catalog # 17-374

Lot # 30442

#### 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 MgCl<sub>2</sub>.

##### **Activator Solution**

Catalog # 20-268, Lot # 31739.

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 # 30423.

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

##### **HDAC Assay Standard, Colorimetric Detection**

Catalog # 12-562, Lot # 30424.

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 # 30044.

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**

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#### Kit Description

**Quantity:** Sufficient reagents for 96 assays per kit.

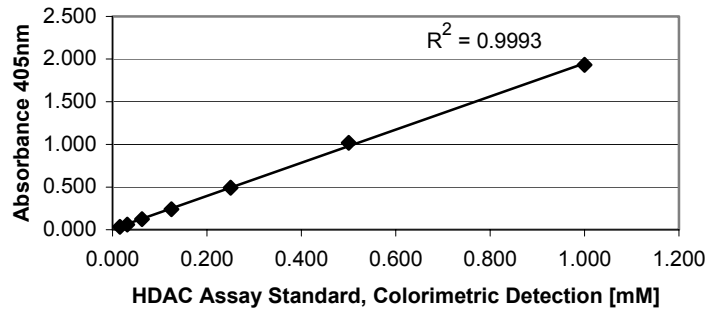
**Storage:** Upon receipt, see individual components for storage conditions. The half volume 96 well plate should be stored at room temperature. All other components are stored at -20°C.

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

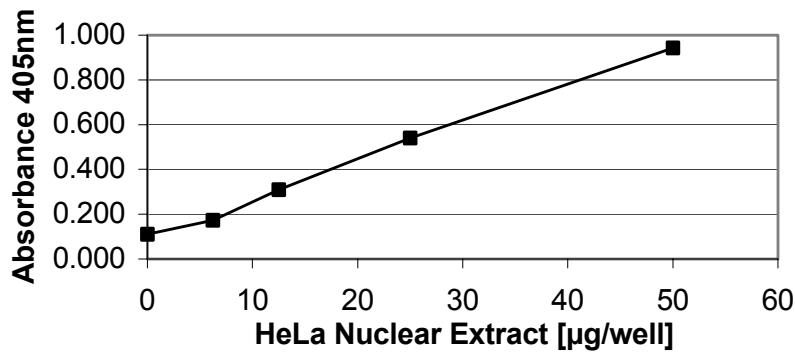
**Use:** This assay is a simple two-step procedure performed in a microtiter plate. In the first step, samples are incubated with the HDAC assay substrate, allowing deacetylation of the substrate. Next, the Activator Solution releases p-nitroanilide from the deacetylated substrate or standard.

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

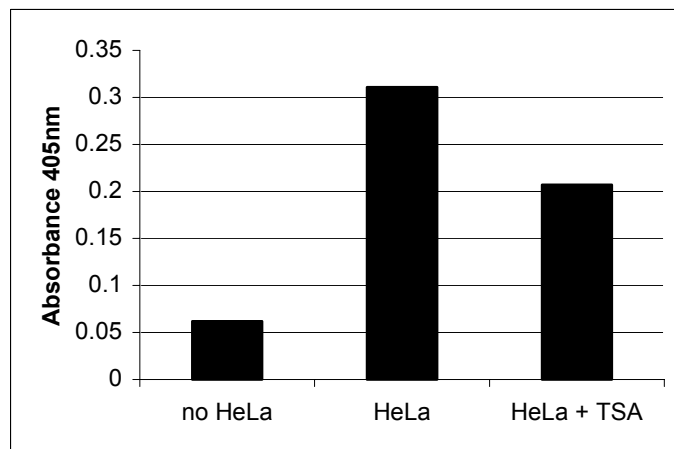
### Quality Control Testing



**Figure 1. HDAC Assay Standard Curve.** 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.** 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.** 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.