Development of Histone Deacetylase Activity Assay Using a Novel Long Wavelength Fluorogenic Substrate

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Introduction

Histone deacetylase (HDAC) enzymes modulate gene expression through the deacetylation of lysine residues on histones, the major proteins of chromatin, and act as transcriptional repressors of genes. Based on their role in cell cycling, apoptosis and differentiation, HDACs have been chosen as therapeutic targets for the treatment of cancer and neurodegenerative diseases. A previously described HDAC assay is based on a substrate generating a shorter wavelength fluorophore, AMC (7-amino-4-methylcoumarin). AMC detection at Ex/Em=350/440 nm shows interference from autofluorescence of components.

To facilitate HDAC drug discovery, we developed an assay using a novel fluorogenic substrate. The substrate, after incubation with HDAC-containing samples and the HDAC developer, releases the green fluorophore and fluorescence can be detected at Ex/Em=490/520 nm. This novel substrate is equally efficient in 2-step or 1-step protocol for measuring HDAC activity.

The SensoLyte® 520 HDAC substrate is highly sensitive and is cell-permeable. It has been validated using HeLa nuclear cell extract enriched with HDACs, with purified HDAC enzymes and in a cell-based system. Long wavelength spectra and higher extinction coefficient of the SensoLyte® 520 HDAC substrate provide less interference from compounds and cell components.

Assay Principle

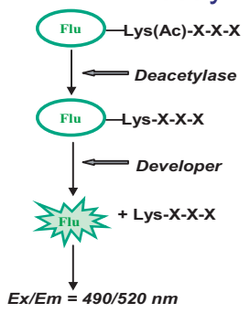

Figure 1. For a 2-step protocol, the substrate is first incubated with HDAC-containing sample. After deacetylation, the addition of the developer releases the fluorophore. For a 1-step protocol, the reaction can be initiated by mixing the substrate, enzyme and developer simultaneously. The release of fluorophore is monitored over time at Ex/Em=490/520 nm.

Materials and Methods

- SensoLyte® 520 HDAC Activity Assay Kit (Cat# 72084)
- HDAC substrate, Ex/Em=490/520 nm upon cleavage
- Recombinant Human HDAC6, HDAC8 (Enzo Life Sciences, Plymouth Meeting, PA)
- HDAC AMC substrate, Ex/Em=354 nm/442 nm upon cleavage
- Commercial HDAC substrate, Ex/Em=350 nm/530 nm upon cleavage (Enzo Life Sciences)

All incubations were performed at 37C in 96-well black opaque plates. Fluorescence was measured using FlexStation 384II (Molecular Devices, Sunnyvale, CA).

Results

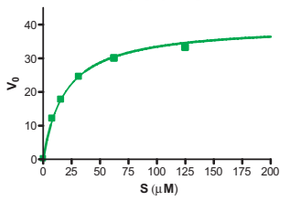

Figure 2. Michaelis-Menten plot for the SensoLyte® HDAC 520 substrate incubated with HeLa nuclear extract. Initial velocities (Vo) were calculated and plotted against substrate concentration. The calculated Km value was 19.62 μM.

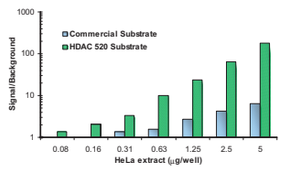

Figure 3. The SensoLyte® 520 HDAC substrate provides higher sensitivity and better linear range than an existing commercial substrate. HDAC substrates at a final concentration of 25 μM were incubated with HeLa nuclear extracts, followed by an additional 15 min incubation with a Trichostatin A-containing developer.

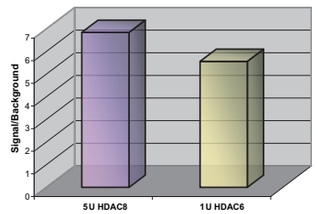

Figure 4. Validation with purified HDAC enzymes. HDAC8 was incubated with 250 μM of SensoLyte® 520 HDAC substrate and HDAC6 was incubated with 12.5 μM of the same substrate for 30 min. Signal was measured after addition of Trichostatin A-containing developer.

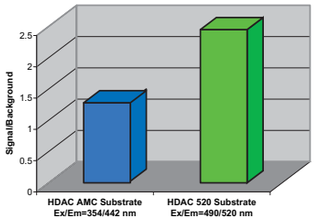

Figure 5. The HDAC 520 substrate can be used in a cell-based assay. High Ex/Em wavelength provides less interference from cell components and allows detection in phenol red-containing media. HeLa cells were incubated with HDAC substrates added to cell culture media and the signal was measured after addition of Trichostatin A-containing developer.

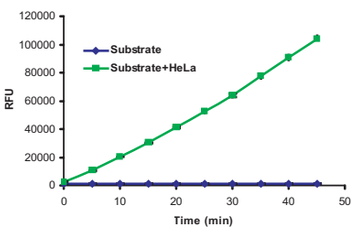

Figure 6. The SensoLyte® 520 HDAC substrate is efficient in 1-step method. In the 1-step protocol, substrate was added to HeLa nuclear extract simultaneously with developer and the reaction monitored over time.

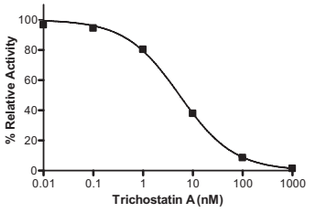

Figure 7. Trichostatin A inhibition of HDAC activity in HeLa nuclear extract measured with SensoLyte® 520 HDAC Activity Assay Kit. The calculated IC50 was 5.4 nM.

Conclusions

- We have developed the SensoLyte® 520 HDAC Activity Assay Kit, an assay based on a highly sensitive cell-permeable fluorogenic substrate.
- The detection of fluorescence at long excitation/emission wavelengths minimizes interference from cell components and cell culture media.
- This SensoLyte® 520 HDAC assay kit is capable of continuous, homogeneous monitoring of the enzymatic reaction in one-step protocol.
- The SensoLyte® 520 HDAC assay kit works in cell-based assay as well as with purified HDAC enzymes.