# FLUOR DE LYS® HDAC6 fluorometric drug discovery kit

A FLUOR DE LYS® fluorescent assay system. The HDAC6 Fluorescent Activity Assay/Drug Discovery Kit is a complete assay system designed to measure the lysyl deacetylase activity of the recombinant human HDAC6 included in the kit. The kit is ideal for chemical library screening for candidate inhibitors or activators or kinetic assay of the enzyme under varying conditions. The FLUOR DE LYS® HDAC6 assay is based on the FLUOR DE LYS® Substrate and FLUOR DE LYS® Developer combination. The assay procedure has two steps. First, the FLUOR DE LYS® Substrate, which comprises an acetylated lysine side chain, is incubated with HDAC6. Deacetylation of the substrate sensitizes the substrate so that, in the second step, treatment with the FLUOR DE LYS®Developer produces a fluorophore.

HDAC6 is a class II HDAC, a group defined by its member's homology to the yeast HDAC. HDAC6 falls into the class IIb subclass, along with HDAC10, but is unique among human HDACs in that it contains two full deacetylase domains. Primarily located to cytoplasm, HDAC6 is a tubulin deacetylase. Other HDAC6 substrates include Hsp90, cortactin and peroxiredoxins. Through these deacetylase activities and its ubiquitin and other binding activities, HDAC6 plays key regulatory roles in cell motility, protein folding, the ubiquitin-proteasome pathway, the aggresome pathway and autophagy, suggesting a central function in the coordination of cellular stress responses. HDAC6 is required for efficient oncogenic tumorigenesis. The mechanisms underlying this requirement may include HDAC6-mediated promotion of invasive cell motility, resistance to anoikis, removal of toxic misfolded proteins, inhibition of Hsp90 stabilization of metastasis suppressors and promotion of angiogenesis. HDAC6 null-mice, while outwardly normal, have increased resistance to chemical carcinogenesis. It is thus reasonable to suppose that HDAC6 inhibition may be a key factor in the anti-cancer effects of pan-HDAC inhibitors and that selective HDAC6 inhibitors may have the potential for greater effectiveness and reduced side-effects.

**Citations:** 35 View Online »

## Ordering Information Order Online »

- Useful for inhibitor screening or characterizing enzyme kinetics
- Includes optimal substrate selected from a panel of acetylated sites in p53 and histones
- Supplied with enough recombinant enzyme for 96 assays (1 x 96-well plate)

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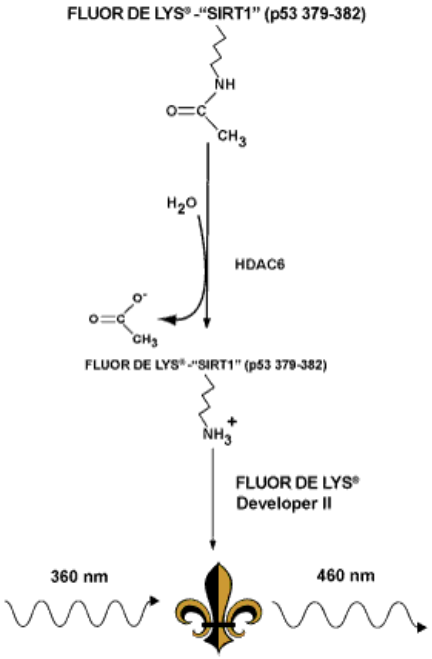
| **BML-AK516-0001** | 96 wells |

## Manuals, SDS & CofA

View Online »



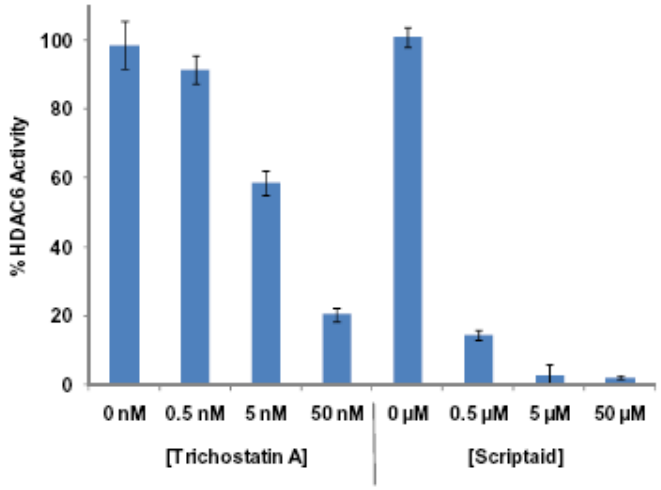




**Figure 3:** Trichostatin A and Scriptaid (Prod. No. BML-GR326) inhibition of HDAC6 determined by FLUOR DE LYS®-SIRT1 (Prod. No. BML-KI177) substrate deacetylation. **Method:** HDAC6 enzyme (400 ng/well) was incubated (37°C) with 12µM substrate at the indicated concentrations of Trichostatin A and Scriptaid. Reactions were stopped after 60min. with FLUOR DE LYS® Developer II and the fluorescence was measured.

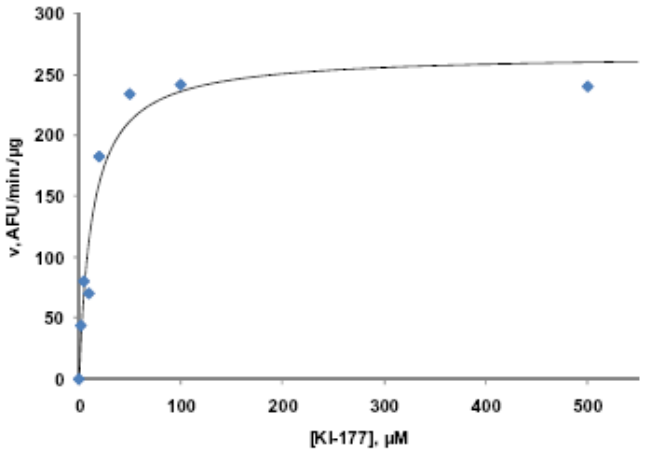



**Figure 2:** Kinetics of FLUOR DE LYS® SIRT1 (Prod. No. BML-KI177) substrate deacetylation by HDAC6. **Method:** HDAC6 enzyme (500 ng/well) was incubated (37°C) with indicated concentrations of substrate. Reactions were stopped after 60min. with FLUOR DE LYS® Developer II and fluorescence was measured. Points are the mean of two independent determinations, each comprising three replicates. Line is a non-linear least squares fit of the data to the Michaelis-Menten equation. The best-fit $K_m$ was 13.0µM and the $V_{max}$ 267 AFU/min/µg.

## Handling & Storage

**Use/Stability** Store all components except the microplate at -80°C for the highest stability. The HDAC6 Enzyme (Prod. No. BML-SE508), must be handled with particular care in order to retain maximum enzymatic activity. Defrost it quickly in a RT water bath or by rubbing between fingers, then immediately store on an ice bath. The remaining unused enzyme should be refrozen quickly, by placing at -80°C. If possible, snap freeze in liquid nitrogen or a dry ice/ethanol bath. To minimize the number of freeze/thaw cycles, aliquot the enzyme into separate tubes and store at -80°C.

**Long Term Storage** -80°C

**Shipping** Dry Ice

## Regulatory Status

RUO - Research Use Only

## Product Details

**Alternative Name** Histone deacetylase 6 fluorescent assay kit

**Application** Activity assay, Fluorescent detection, HTS

**Contents**

**HDAC6** (Histone Deacetylase 6) (human, recombinant) (Prod. No. BML-SE508)
(50 µg; 10mM TRIS, pH 7.5, 100mM NaCl, 3mM MgCl2, 10% glycerol)
Storage: -80°C, avoid freeze/thaw cycles!
**FLUOR DE LYS® SIRT1, Deacetylase Substrate** (Prod. No. BML-KI177)
(100µl; 5mM solution in 50mM TRIS/Cl, pH 8.0, 137mM NaCl, 2.7mM KCl, 1mM MgCl2)
Storage: -80°C
**FLUOR DE LYS® Developer II Concentrate (5x)** (Prod. No. BML-KI176)
(5 x 250 µl; 5x Stock Solution; Dilute in Assay Buffer before use
Storage: -80°C
**Trichostatin A** (HDAC Inhibitor) (Prod. No. BML-GR309-9090)
(100 µl; 0.2mM in DMSO)
Storage: -80°C
**FLUOR DE LYS® Deacetylated Standard** (Prod. No. BML-KI142)
(30 µl; 10mM in DMSO)
Storage: -80°C
**HDAC Assay Buffer II** (Prod. No. BML-KI422)
(20ml; 50mM TRIS/Cl, pH 8.0, 137mM NaCl, 2.7mM KCl, 1mM MgCl2, 1mg/ml BSA)
Storage: -20°C
**HDAC Assay Buffer** (Prod. No. BML-KI143)
(20ml; 50mM TRIS/Cl, pH 8.0, 137mM NaCl, 2.7mM KCl, 1mM MgCl2)
Storage: -20°C
**1/2 volume microplate** (Prod. No. BML-KI101)
Storage: Ambient
**1/2 volume white NBS microplate** (Prod. No. BML-KI584)
Storage: Ambient

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| **UniProt ID** | Q9UBN7 |


ENZO LIFE SCIENCES, INC.
Phone: 800.942.0430
info-usa@enzolifesciences.com

European Sales Office
ENZO LIFE SCIENCES (ELS) AG
Phone: +41 61 926 8989
info-eu@enzolifesciences.com

Belgium, The Netherlands & Luxembourg
Phone: +32 3 466 0420
info-be@enzolifesciences.com

France
Phone: +33 472 440 655
info-fr@enzolifesciences.com

Germany
Phone: +49 7621 5500 526
info-de@enzolifesciences.com

UK & Ireland
Phone (UK customers): 0845 601 1488
Phone: +44 1392 825900
info-uk@enzolifesciences.com



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