



HDAC & Sirtuin Assay Kits

The Fluor de Lys[®] Assay System

- Versatile, HTS-friendly fluorometric assays
- Detect activity from purified enzymes, immunoprecipitates, cell & tissue lysates or whole cells
- For class I, IIb & IV HDACs and sirtuins

The *Fluor de Lys*[®] (Fluorescent deacetylation of lysine) assay system was developed for simple, nonradioactive measurement of deacetylase activity. Deacetylation of a *Fluor de Lys*[®] substrate by a source of deacetylase activity, including purified enzyme or enzyme complex, cell lysate or whole cells, sensitizes the substrate so that, in a second step, treatment with the appropriate *Fluor de Lys*[®] developer produces a fluorophore (see Figure). The assay is compatible with class I, IIb & IV HDACs and a variety of substrates are available based on acetylated sites found in p53 and histones. *Fluor de Lys*[®] substrate (Prod. No. BML-KI104) is cell-permeable and allows cell-based determination of HDAC activity (Prod. No. BML-AK503). This system is the basis of several activity assays/drug discovery kits for HDACs (Prod. No. BML-AK500, BML-AK511, BML-AK516, BML-AK518, BML-AK531) and sirtuins (Prod. No. BML-AK555, BML-AK556 and BML-AK557).

Selected Citations Using the *Fluor de Lys*[®] Activity Assays:

X. Zhou et al.; PNAS **98**, 10572 (2001) • K.J. Bitterman et al.; J. Biol. Chem. **277**, 45099 (2002) • B. Heltweg and Jung, M.; Anal. Biochem. **302**, 175 (2002) • K. Ito et al.; PNAS **99**, 8921 (2002) • S. Milutinovic et al.; J. Biol. Chem. **277**, 20974 (2002) • R.M. Anderson et al.; Science **302**, 2124 (2003) • K.T. Howitz et al.; Nature **425**, 191 (2003) • G.V. Kapustin et al.; Org. Lett. **5**, 3053 (2003) • D.K. Kim et al.; J. Med. Chem. **46**, 5745 (2003) • C.G. Kleer et al.; PNAS **100**, 11606 (2003) • K. Zhao et al.; Nat. Struct. Biol. **10**, 864 (2003) • T. Suzuki et al.; Bioorg. Med. Chem. Lett. **13**, 4321 (2003) • B.G. Cosio et al.; Am. J. Respir. Crit Care Med. **170**, 141 (2004) • C.M. Gallo et al.; Mol. Cell Biol. **24**, 1301 (2004) • N. Gurvich et al.; Cancer Res. **64**, 1079 (2004) • L.H. Wang et al.; Nat. Med. **10**, 40 (2004) • J.G. Wood et al.; Nature **430**, 686 (2004) • F. Yeung et al.; EMBO J. **23**, 2369 (2004) • J.L. Avalos et al.; Mol. Cell **17**, 855 (2005) • K. Ito et al.; N. Engl. J. Med. **352**, 1967 (2005) • A. Mai et al.; J. Med. Chem. **48**, 7789 (2005) • E. Michishita et al.; Mol. Biol. Cell **16**, 4623 (2005) • A.D. Napper et al.; J. Med. Chem. **48**, 8045 (2005) • T. Suzuki et al.; J. Med. Chem. **48**, 1019 (2005) • P. Aksoy et al.; BBRC **349**, 353 (2006) • V.C. de Boer et al.; Mech. Ageing Dev. **127**, 618 (2006) • S.L. Gantt et al.; Biochemistry **45**, 6170 (2006) • W. Gu et al.; Bioorg. Med. Chem. **14**, 3320 (2006) • D. Herman et al.; Nat. Chem. Biol. **2**, 551 (2006) • X. Li et al.; Cancer Res. **66**, 9323 (2006) • V.M. Nayagam et al.; J. Biomol. Screen. **11**, 959 (2006) • J.M. Solomon et al.; Mol. Cell Biol. **26**, 28 (2006) • P.H. Kiviranta et al.; Bioorg. Med. Chem. Lett. **17**, 2448 (2007) • T.F. Outeiro et al.; Science **317**, 516 (2007) • S. Lain et al.; Cancer Cell **13**, 454 (2008) • B. Jung-Hynes et al.; J. Biol. Chem. **284**, 3823 (2009)

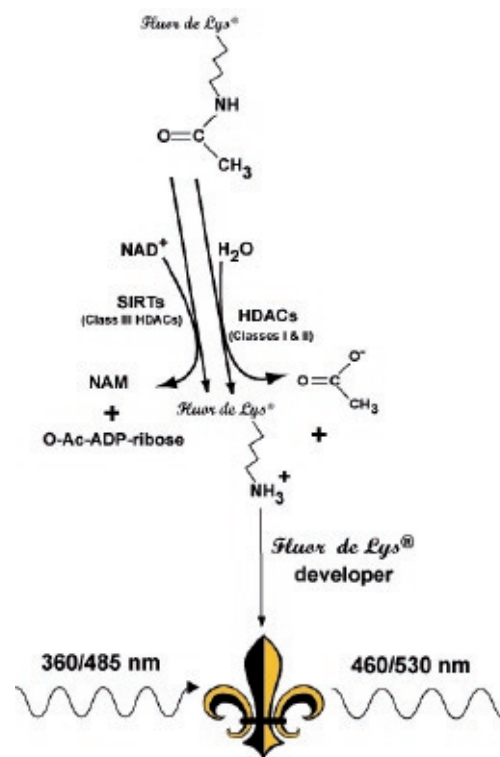


FIGURE: Mechanism of the *Fluor de Lys*[®] HDAC assay. Deacetylation of the substrate sensitizes it to the developer, which then generates a fluorophore (symbol).

Just released: *Fluor de Lys*[®]-green – A superior substrate for deacetylase assays
For details see page 2.

General *Fluor de Lys*[®] Activity Assays

- Useful for assaying lysates, immunoprecipitates or inhibitor screening
- Includes HeLa nuclear extract, a rich source of HDACs 1, 2 & 3, for use as a positive control or as a source of HDAC activity for screening
- Compatible with class I, IIb & IV HDACs and sirtuins (with addition of NAD⁺)
- Includes enough reagent for 100-200 assays



Product	Prod. No.	Size
<i>Fluor-de-Lys</i> [®] HDAC fluorometric activity assay kit	BML-AK500-0001	1 Kit
<i>Color-de-Lys</i> [™] HDAC colorimetric activity assay kit	BML-AK501-0001	1 Kit

Fluor de Lys[®]-Green

NEW

- Increased sensitivity
- Longer wavelength excitation/emission avoids interference from auto-fluorescence of cell constituents and test compounds
- First in a series of new substrates and fluorophores for the most flexible HDAC assay system available

The *Fluor de Lys*[®]-green HDAC assay is an HTS-compatible, homogenous assay for measuring histone deacetylase (HDAC) or sirtuin activity from cell or nuclear extracts, immunoprecipitates or purified enzymes. The assay employs *Fluor de Lys*[®]-green, a new substrate offering higher sensitivity and an excitation and emission (485/530nm) that avoids quenching and fluorescent interference from compounds absorbing in the near UV and blue range.

Fluor-de-Lys[®]-green HDAC fluorometric activity assay kit

BML-AK530-0001

1 Kit

Contains HeLa nuclear extract for use as a source of HDAC activity or positive control, *Fluor de Lys*[®]-green substrate and developer, NAD⁺ and nicotinamide for sirtuin assays, *Fluor de Lys*[®]-green deacetylated standard, assay buffer, the known HDAC inhibitor Trichostatin A, 1/2-vol. 96-well plates, and detailed instructions.

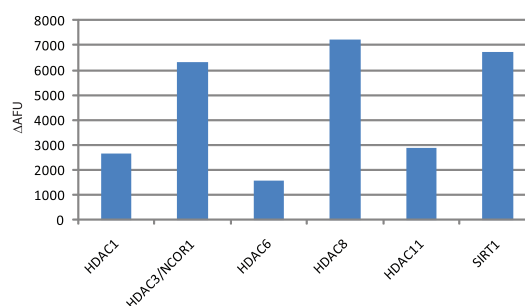


FIGURE: Class I, IIb & IV HDACs and sirtuins deacetylate the *Fluor de Lys*[®]-green substrate.

Fluor de Lys[®] Drug Discovery Assays

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

Each kit includes: Recombinant human enzyme, optimal *Fluor de Lys*[®]-substrate and developer, NAD⁺ (sirtuin assays only), assay buffer, control inhibitor, white and clear 1/2-vol. 96-well plates, detailed instructions. Enough reagent is included for 96-192 assays.

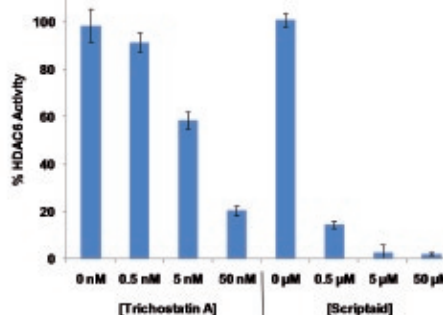


FIGURE: Trichostatin A (Prod. No. BML-GR309) and Scriptaid (Prod. No. BML-GR326) inhibition of HDAC6 determined using the HDAC6 drug discovery assay (Prod. No. BML-AK516).

Product	Prod. No.	Size
<i>Fluor-de-Lys</i> [®] HDAC1 fluorometric drug discovery assay kit	BML-AK511-0001	1 Kit
<i>Fluor-de-Lys</i> [®] HDAC3/NCOR1 fluorometric drug discovery assay kit	BML-AK531-0001	1 Kit
<i>Fluor-de-Lys</i> [®] HDAC6 fluorometric drug discovery assay kit	BML-AK516-0001	1 Kit
<i>Fluor-de-Lys</i> [®] HDAC8 fluorometric drug discovery assay kit	BML-AK518-0001	1 Kit
<i>Fluor-de-Lys</i> [®] SIRT1 fluorometric drug discovery assay kit	BML-AK555-0001	1 Kit
<i>Fluor-de-Lys</i> [®] SIRT2 fluorometric drug discovery assay kit	BML-AK556-0001	1 Kit
<i>Fluor-de-Lys</i> [®] SIRT3 fluorometric drug discovery assay kit	BML-AK557-0001	1 Kit

Fluor de Lys[®] Cellular HDAC Assay

- Allows determination of deacetylase activity within an undisturbed cellular environment
- Provides accurate activity information reflective of endogenous regulation
- Allows detection of inhibitors or activators that act indirectly to affect deacetylase activity
- Suitable for high-throughput cell-based deacetylase assays

Fluor-de-Lys[®] HDAC fluorometric cellular activity assay kit

BML-AK503-0001

1 Kit

The *Fluor-de-Lys*[®] HDAC fluorometric cellular activity assay kit utilizes the cell permeable *Fluor de Lys*[®] substrate (Prod. No. BML-K1104) to provide a straightforward approach for measuring deacetylase activity in cultured cells. The deacetylated substrate accumulates inside cells and deacetylation can be quantitated by addition of developer to lysed cells and subsequent measurement of fluorescence.

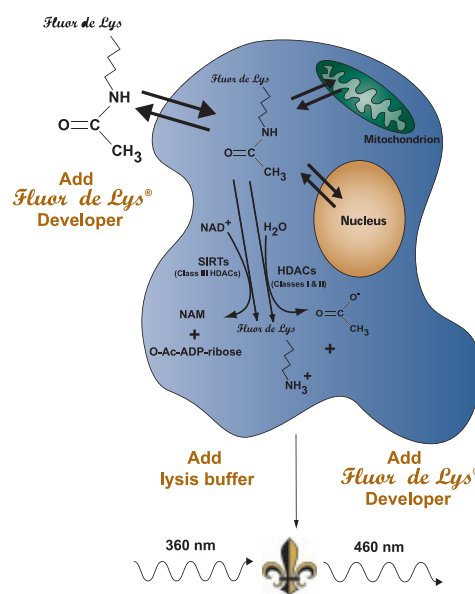


FIGURE: Mechanism of the *Fluor de Lys*[®] HDAC fluorometric cellular activity assay.

Deacetylase Enzymes & Substrates



Product	Prod. No.	Size
HDAC (rat)	ALX-202-052-L002	2 ml
HDAC1 (human), (rec.) (His-tag)	BML-SE456-0050	50 µg
HDAC2 (human) (1-488), (rec.) (His-tag)	BML-SE500-0050	50 µg
HDAC2 (human) (full-length), (rec.) (His-tag)	BML-SE533-0050	50 µg
HDAC3 (human), (rec.) (His-tag)	BML-SE507-0050	50 µg
HDAC3/NCOR1 complex (human), (rec.)	BML-SE515-0050	50 µg
HDAC6 (human), (rec.) (His-tag)	BML-SE508-0050	50 µg
HDAC8 (human) , (rec.) (His-tag)	BML-SE145-0100	100 U
HDAC10 (human), (rec.) (His-tag)	BML-SE559-0050	50 µg
HDAC11 (human), (rec.) (His-tag)	BML-SE560-0050	50 µg
SIRT1 (human), (rec.) (His-tag)	BML-SE239-0100	100 U
SIRT2 (human), (rec.) (His-tag)	BML-SE251-0500	500 U
SIRT3 (human), (rec.) (His-tag)	BML-SE270-0500	500 U
SIRT5 (human), (rec.) (His-tag)	BML-SE555-0050	500 µg

Substrates and Developers	Sequence source (a.a.)	Sequence	Prod. No.	Size
<i>Fluor-de-Lys</i> [®] deacetylase substrate*	ε-acetyl-lysine	K(Ac)	BML-KI104-0050	50 µl
<i>Fluor-de-Lys</i> [®] -green*	ε-acetyl-lysine	K(Ac)	BML-KI572-0050	50 µl
<i>Fluor-de-Lys</i> [®] -H4-AcK16*	Histone H4 (12-16)	KGGA(K)Ac	BML-KI174-0005	0.5 µmol
<i>Fluor-de-Lys</i> [®] -HDAC8**	p53 (379-382)	RHK(Ac)K(Ac)	BML-KI178-0005	0.5 µmol
<i>Fluor-de-Lys</i> [®] -SIRT1**	p53 (379-382)	RHKK(Ac)	BML-KI177-0005	0.5 µmol
<i>Fluor-de-Lys</i> [®] -SIRT2**	p53 (317-320)	QP(K)Ac	BML-KI179-0005	0.5 µmol
<i>Fluor-de-Lys</i> [®] developer concentrate			BML-KI105-0300	300 µl
<i>Fluor-de-Lys</i> [®] developer II			BML-KI176-1250	1.25 ml

Other substrates based on acetylated sites can be produced on a custom basis. Contact customquote-usa@enzolifesciences.com.

*Must be used in conjunction with *Fluor de Lys*[®] developer concentrate (Prod. No. BML-KI105)

**Must be used in conjunction with *Fluor de Lys*[®] developer II (Prod. No. BML-KI176)

Substrate Preferences for HDAC & Sirtuin Enzymes

Substrate	HDAC								HeLa nuclear extract	Sirtuin				
	1	2	3	6	8	10	11	1		2	3	5		
<i>Fluor de Lys</i> [®]	+++	++	++++	++	+	++++	++	++	+	+	+	+		
<i>Fluor de Lys</i> [®] -green	++	++++	++++	+	++++	+	++	++++	++++	++	++	+		
<i>Fluor de Lys</i> [®] -H4-AcK16	+++	+++	++++	+++++	+++	n.d.	+++	+++	++++	+++	+	+++		
<i>Fluor de Lys</i> [®] -HDAC8	++++	+++++	++++	+++++	+++++	n.d.	+++++	+++++	+++	++++	+++	+++++		
<i>Fluor de Lys</i> [®] -SIRT1	+++++	+++++	+++++	+++++	+	+++++	+++++	+++++	+++++	+++++	+++	+++++		
<i>Fluor de Lys</i> [®] -SIRT2	+++	++++	++++	++++	++	+	+	++++	++	+++++	+++++	++++		
<i>Fluor de Lys</i> [®] Substrate concentration	5µM	5µM	50µM	50µM	25µM	25µM	5µM	25µM	25/500µM	25µM	10µM	500µM		

TABLE 1: Substrate preferences for a number of HDACs and sirtuins. The table indicates the substrate preferences for the indicated deacetylase among a panel of *Fluor de Lys*[®] substrates based on short stretches of human histones H3, H4 and p53 sequence (see product listing above). The concentration of each substrate is indicated in the last row. For sirtuins the concentration of the co-substrate NAD⁺ was 500µM. Class IIa enzymes (HDACs 4, 5, 7 and 9) were not included because they exhibit very little activity with standard peptide substrates (see Unraveling the hidden catalytic activity of vertebrate class IIa histone deacetylases: A. Lahm et al.; PNAS **104**, 17335 (2007)). They do however have low but measurable activity with *Fluor de Lys*[®]-SIRT2 (Prod. No. BML-KI179). n.d. indicates not determined.

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