

Fluor de Lys[®]-SIRT1, Deacetylase Substrate

CATALOG NO.: BML-KI177

LOT NO.: TEMP

DESCRIPTION: *Fluor de Lys*[®]-SIRT1 is a fluorogenic, acetylated peptide substrate for SIRT1 (human Sirtuin 1). Based on residues 379-382 of p53 (Arg-His-Lys-Lys(Ac)), a site of regulatory acetylation by the p300 and CBP acetyltransferases (lysines 381, 382)¹⁻⁶, it was the best for SIRT1 from among a panel of substrates patterned on p53, histone H3 and histone H4 acetylation sites⁷. *Fluor de Lys*[®]-SIRT1 is deacetylated by SIRT1 (Cat. # BML-SE239) at a rate of more than 8-fold that of the acetylated lysine substrate, *Fluor de Lys*[®] (Cat. # BML-KI104; acetylated substrates both at 25 μ M, 500 μ M NAD⁺)⁷. The K_m of *Fluor de Lys*[®]-SIRT1 for human recombinant Sirtuin 1 (SIRT1, Cat. #BML-SE239) is 108 μ M (determined at 37°C, 500 μ M NAD⁺)⁷. Must be used in conjunction with *Fluor de Lys*[®] Developer II (Cat. # BML-KI176; see attached assay conditions). Fluorescent signal indicates deacetylation of Lys-382.

--
PURITY: >95% by HPLC.

APPLICATIONS: Study kinetics, inhibitor sensitivity and substrate specificity of SIRT1. Ideal for drug discovery and HTS applications.

SUPPLIED AS: A 5 mM solution in 50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂

QUANTITY: 0.5 μ mol (100 μ l, 5 mM)

STORAGE: -20°C or 70°C.

REFERENCES:

1. W. Gu and R.G. Roeder *Cell* 1997 **90** 595
2. K. Sakaguchi *et al. Genes Dev.* 1998 **12** 2831
3. L. Liu *et al. Mol. Cell. Biol.* 1999 **19** 1202
4. A. Ito *et al. EMBO J.* 2001 **20** 1331
5. N.A. Barlev *et al. Mol. Cell* 2001 **8** 1243
6. A. Ito *et al. EMBO J.* 2002 **21** 6236
7. BIOMOL Research Laboratories, Inc. *unpublished results*

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

**Assay of SIRT1 (Cat. # BML-SE239) with
Fluor de Lys[®]-SIRT1 (BML-KI177) & *Fluor de Lys*[®] Developer II (BML-KI176)**

Components of Assay:

HDAC Assay Buffer (BML-KI143*, for dilution of Developer II Concentrate, preparation of Nicotinamide stock)
(50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂)

SIRT1 Assay Buffer (BML-KI143* supplemented with BSA)
(50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 1 mg/ml BSA)

SIRT1 (BML-SE239)
Dilute to 0.2 U/μl with SIRT1 Assay Buffer, enough for one day's assay and keep on ice.

Fluor de Lys[®]-SIRT1 (BML-KI177)
Dilute 5 mM stock in HDAC Assay Buffer.

NAD⁺ (β-nicotinamide adenine dinucleotide, oxidized form)
Prepare a 10 mM stock in SIRT1 Assay Buffer, store frozen at -20°C.

2x Substrates Solution
Prepare a combined 2x substrates solution, with *Fluor de Lys*[®]-SIRT1 at 200 μM and NAD⁺ at 1 mM. For example, prepare 1 ml by mixing 100 μl 10 mM NAD⁺, 40 μl 5 mM *Fluor de Lys*[®]-SIRT1 and 860 μl SIRT1 Assay Buffer. Warm to 37°C before use.

Nicotinamide (Sirtuin inhibitor)
Prepare 50 mM and 10 mM stocks respectively in HDAC Assay Buffer and SIRT1 Assay Buffer. May be stored at -20°C.

Fluor de Lys[®] Developer II (5x Concentrate, Cat. # BML-KI176)
Shortly before use, dilute 5x stock solution to 1x plus 2 mM nicotinamide. For example, prepare 1 ml by mixing 200 μl of the 5x Concentrate, 760 μl HDAC Assay Buffer (BML-KI143) and 40 μl 50 mM nicotinamide. Store the 1x Developer II plus nicotinamide on ice until use. Do not store excess, but prepare freshly as needed.

Fluor de Lys[®] Deacetylated Standard (BML-KI142)*
Dilute the 10 mM stock in DMSO to 1 μM with SIRT1 Assay Buffer.

½ Volume 96-well white micro-plate*

*Components of the HDAC *Fluor de Lys*[®] Fluorescent Activity Assay (Cat. # BML-AK500), which are also sold separately.

Reaction Condition Examples :

- 1) Designate wells for four reactions: 30 min rxn; 30 min rxn plus nicotinamide; 0 min rxn and a Standard well.
- 2) Add 20 μl of SIRT1 Assay Buffer to the 30 min rxn well and the 0 min rxn well. To the third well (30 min. plus nicotinamide) add 2.5 μl of 10 mM nicotinamide plus 17.5 μl of SIRT1 Assay Buffer. Allow to equilibrate to assay temperature (37°C). (Leave Standard well empty until step 7).
- 3) Add 5 μl of diluted SIRT1 (BML-SE239, 0.2 U/μl) to the wells for the 0 min, 30 min, and 30 min. plus nicotinamide rxns.
- 4) To start reactions, add 25 μl of the 2x Substrates (37°C) to both 30 min reaction wells. Final [NAD⁺] will be 500 μM and final [*Fluor de Lys*[®]-SIRT1] will be 100 μM. Allow reactions to run 30 min @ 37°C.
- 5) Add 50 μl of 1x Developer II plus nicotinamide to both 30 min. reaction wells.
- 6) To the 0 min rxn well, add 50 μl of 1x Developer II plus nicotinamide, immediately followed by 25 μl of substrate.
- 7) For the standard, mix 50 μl of 1 μM standard with 50 μl of 1x Developer II plus nicotinamide in the fourth well.
- 8) Allow 45 min. at 37°C for signal to develop and then read plate in a microplate-reading fluorimeter capable of excitation at a wavelength in the range of 350-380 and detection of emitted light in the range of 440-460 nm.

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

PRODUCT DATA SHEET



- 9) Data analysis: Determine the Δ AFU (Arbitrary Fluorescence Units) for the two 30 min rxns. (AFU of 30 min rxn. (with or without nicotinamide) minus AFU of 0 min rxn). Determine AFU/pmol by dividing the Deacetylated standard reading (AFU) by 50 pmol. Calculate pmol of substrate deacetylated in 30 min (divide Δ AFU by AFU/pmol).

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.