PRODUCT DATA SHEET

Fluor de Lys®–Green Substrate

CATALOG NO.: BML-KI572 **LOT NO.:** TEMP

DESCRIPTION: The *Fluor de Lys*®–Green substrate offers a highly sensitive and convenient alternative to radiolabeled, acetylated histones or peptide/HPLC methods for the assay of histone deacetylases. The assay procedure has two steps. First, the *Fluor de Lys*®-Green Substrate, which comprises an acetylated lysine side chain, is incubated with a sample containing HDAC activity (HeLa nuclear or other extract, purified enzyme, bead-bound immunocomplex, etc.). Deacetylation of the substrate sensitizes the substrate so that, in the second step, treatment with the *Fluor de Lys*® Developer (BML-KI105) produces a fluorophore. The *Fluor de Lys*®-Green substrate has a higher wavelength excitation and emission than the original *Fluor de Lys*® substrate (BML-KI104). This avoids interference by quenching or fluorescence from compounds absorbing and/or emitting in the near UV and blue range.

NOTE: Must be used in conjunction with *Fluor de Lys*® Developer (Cat. # BML-KI105; see attached assay conditions).

PURITY: >95% by HPLC.

APPLICATIONS: Study of HDAC or sirtuin kinetics, regulation and inhibitor sensitivity. Ideal for drug discovery and HTS applications. Cell-based HDAC assays.

SUPPLIED AS: A 50 mM solution in DMSO (dimethylsulfoxide)

QUANTITY: 2.5 µmol (50 µl, 50 mM)

STORAGE: -20°C or 70°C.

Assay of HDACs and Sirtuins with *Fluor de Lys*®-Green Substrate (BML-KI572) & *Fluor de Lys*® Developer (BML-KI105)

Components of Assay:

HDAC Assay Buffer (BML-KI143)*
50 mM Tris/Cl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, (Supplement with 1 mg/ml BSA for dilution and assay of recombinant HDAC8, Cat. # BML-SE145)

Enzyme Sources
With appropriate modifications virtually any sample containing active HDACs or sirtuins may be assayed. This would include cellular extracts (e.g. HeLa Nuclear Extract, Cat. # BML-KI140), purified and recombinant enzymes (e.g. HDAC8, Cat. # BML-SE145; SIRT1, Cat. #BML-SE239). Dilute samples, as needed, with Assay Buffer such that 5 µl may be used per assay well. For assays on immunoprecipitates and intact cells, consult the product data sheet for the *Fluor de Lys*® HDAC Assay Kit (Cat. # BML-AK500).
NOTE: Assay of sirtuins requires addition of the acetyl-acceptor substrate NAD^+. A sirtuin inhibitor, such as nicotinamide, should be used in place of trichostatin A in the 1X Developer.

Fluor de Lys®-Green Substrate (BML-KI572)*
50 mM stock in DMSO. Thaw at room temperature before use.

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

2X Substrate(s) Solution
For class I or class II HDACs, prepare a 2x solution by diluting the *Fluor de Lys*®-Green Substrate stock (50 mM in DMSO), to, for example, 500 µM. For sirtuin assays, prepare a combined 2X substrates solution by diluting the *Fluor de Lys*®-Green (50 mM) and NAD^+ (10 mM) stocks to, for example, 500 µM and 1 mM, respectively, in Assay Buffer (BML-KI143). (Note that freezing/thawing of *Fluor de Lys*®-Green Substrate solutions in Assay Buffer may cause precipitation of the Substrate).

Trichostatin A (BML-GR309) (HDAC inhibitor for use as 'stop' in 1X Developer and as model inhibitor for '+Inhibitor' samples)
Prepare a 0.2 mM stock in DMSO and a dilution of this stock to 5 µM with Assay Buffer.

Nicotinamide (sirtuin inhibitor for use as 'stop' in 1X Developer and as model inhibitor for '+Inhibitor' samples)
Prepare a 50 mM stock in Assay Buffer and a dilution to 5 mM also in Assay Buffer. Store both frozen at -20°C.

Fluor de Lys® Developer (BML-KI105)*
Shortly before use, dilute 20x stock solution to 1x. (First, dilute the Developer concentrate 20-fold (e.g. 50 µl plus 950 µl Assay Buffer) in cold Assay Buffer. For class I and II HDACs, dilute the 0.2 mM Trichostatin A in DMSO 100-fold in the 1x Developer just prepared (e.g. 10 µl in 1 ml). For sirtuins dilute the 50 mM nicotinamide 50-fold in the 1x Developer just prepared (e.g. 20 µl in 1 ml). Store diluted Developer on ice until use.

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

½ Volume 96-well white micro-plate (BML-KI110)*

*Components of the *HDAC Fluor de Lys*®*-Green Fluorescent Activity Assay* (Cat. # BML-AK530), which are also sold separately.

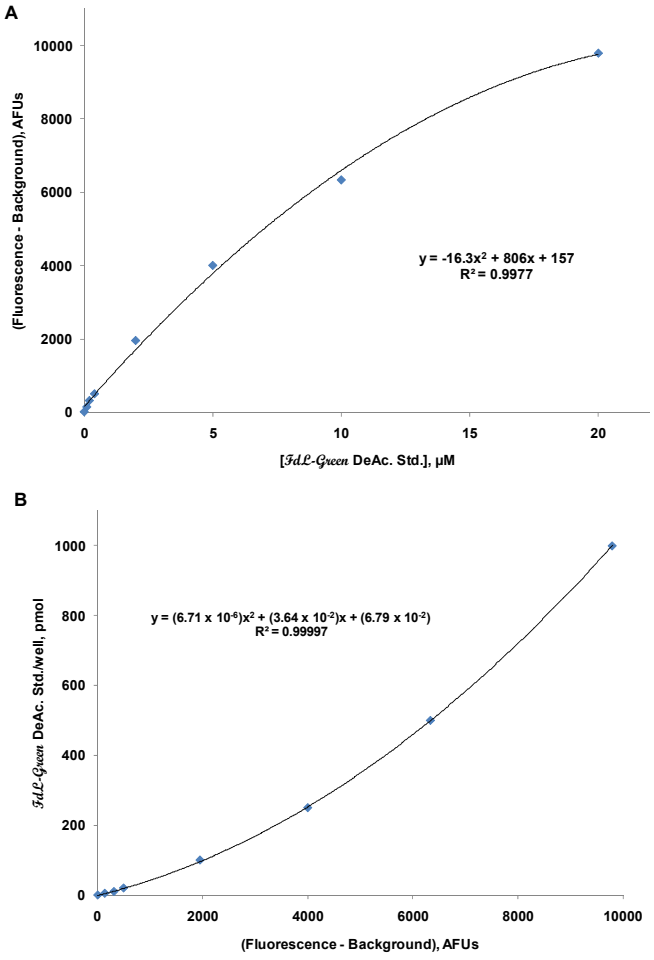
Some Examples of Reaction Conditions:

1) Designate wells for four types of reaction : 'Control'; '+ Inhibitor' (Trichostatin A for class I or II HDACs, nicotinamide for sirtuins); 'Time 0', and 'Standard'.
2) Warm all reagents except the Developer to desired assay temperature (e.g. 37°C).
3) The table below lists the components, and their volumes, for each of the four reaction types. Except for the 'Time 0' sample the reagents may be added in the order listed, from left to right. Timing of the deacetylation reaction for the 'Control' and '+ Inhibitor' samples begins upon addition of the Substrates. The 2X Substrate(s) should be left out of the 'Time 0' well and added only after addition of the Developer (see step 4.).

Sample	Assay Buffer	Enzyme Sample	Nicotinamide (5 mM) or Trichost. A (5 µM)	2x Substrate(s)	DeAc. Std. (10 µM)
Control	20 µl	5 µl	0	25 µl	0
+Inhibitor	15 µl	5 µl	5 µl	25 µl	0
Time 0	20 µl	5 µl	0	25 µl	0
Standard	20 µl	0	0	25 µl	5 µl

4) After the desired deacetylation reaction time (e.g. 60 min.), add 50 ul of 1X Developer (plus trichostatin A or nicotinamide) to each well and mix thoroughly. After this addition of Developer to the 'Time 0' well, add 25 µl of the 2X Substrates, which had been withheld earlier.
5) Incubate the plate 10-15 min. For this step, any temperature in the range of 25-37°C is OK.
6) Read the fluorescence signals in an appropriate microplate-reading fluorimeter, capable of excitation at wavelength in the range of 470-500 nm and detection of emitted light at 520-550nm.
7) Plots of fluorescence (y-axis) versus concentration of the *Fluor de Lys®-Green* Deacetylated Standard (x-axis, *Fdl-Green* DeAc. Std.) and pmol. *Fdl-Green* DeAc. Std. (y-axis) vs. fluorescence are shown in **A** and **B.** Either type of plot fits well to a second-order polynomial. Fit equations

of type **B** may be used to convert fluorescence increases due to deacetylation (sample fluorescence minus time-zero or no-enzyme blank) to the pmols of substrate deacetylated. The accuracy of the fluorescence to pmol conversion may be increased by dividing the standard curve data into several fluorescence ranges and obtaining a best-fit for each. Alternatively, the pmols deacetylated may simply be interpolated from a data set relating pmols of Standard to fluorescence.



diluted in Assay Buffer, to the indicated concentrations, and 50 µl aliquots mixed with 50 µl Developer. Fluorescence was measured in the wells of the white microtiter-plate (BML-KI571) with a CytoFluor™ II fluorescence plate reader (PerSeptive Biosystems, Ex. 485 nm, Em. 530 nm, gain=37). The same data is plotted two ways, in **A** as Fluorescence (minus background) as function of the concentration of *FdL-Green* DeAc. Std. and in **B** as pmols of *FdL-Green* DeAc. Std. per well as a function of Fluorescence (minus background). Best-fit curves to second-order polynomials were obtained with Microsoft XL (equations). The equation from a plot of type **B** may be used to convert fluorescence increases due to deacetylation to the number of pmol of *Fluor de Lys®-Green* Substrate that have been deacetylated.

The difference between the 'Time 0' reading and the 'Standard' reading (1 µM Standard in the 50 µl deacetylation reaction) can be used to calculate a conversion factor (AFU/µM) for relating the fluorescence signal to the decrease in concentration of the acetylated substrate.

Figure. Fluorescence Standard Curves. *Fluor de Lys®-Green* Deacetylated Standard (*FdL-Green* DeAc. Std.) was