Protocol

Assay of HDAC11 (BML-SE560)

with

FLUOR DE LYS®-SIRT1 (BML-KI177)

and

FLUOR DE LYS Developer II (BML-KI176)

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

COMPONENTS OF ASSAY:

HDAC Assay Buffer II (BML-KI422)

(50mM Tris/Cl, pH 8.0, 137mM NaCl, 2.7mM KCl, 1mM$MgCl_2$, 1mg/ml BSA (fatty acid free BSA, e.g. Sigma catalog # A-3803))

HDAC11 (BML-SE560)

Dilute to enough HDAC11 to 0.1µg/µl with HDAC Assay Buffer II (BML-KI422) to provide 5µl per well. Keep on ice until use.

FLUOR DE LYS®-SIRT1 (BML-KI177)

Thaw quickly and keep on ice. Dilute 5mM stock in HDAC Assay Buffer II.

2x Substrate Solution

Prepare a 2x substrate solution by diluting the 5mM **"**FLUOR DE LYS-**SIRT1"** (BML-KI177) in HDAC Assay Buffer II (BML-KI422). Each assay well will require 25µl (see below). For example, prepare 1ml of 20µM substrate (for final 10µM) by mixing 4µl of 5mM FLUOR DE LYS-**SIRT1** (BML-KI177) and 996µl HDAC Assay Buffer II (BML-KI422). Warm to 37°C before use. (**NOTE:** Determinations of HDAC11's K_m for **"**FLUOR DE LYS-**SIRT1"** (BML-KI177) fall in the 5-10µM range. Therefore, a reasonable substrate concentration for inhibitor screening would be 10µM **"**FLUOR DE LYS-**SIRT1"** (BML-KI177), while 200 or 500µM would be more suitable for a specific activity measurement at a saturating substrate concentration.)

Trichostatin A (BML-GR309; HDAC Inhibitor)

Prepare a 0.2mM stock in dimethylsulfoxide (DMSO). DMSO stock may be stored at -20°C. The 0.2mM stock will be diluted 100-fold in 1x Developer II in order to stop HDAC activity at the start of the signal development process. To prepare a stock for use in testing trichostatin inhibition (i.e. for addition to the deacetylation phase of the reaction), dilute 0.2mM stock to 10µM in HDAC Assay Buffer II (e.g. 5µl plus 95µl) and keep on ice. Addition of 2.5µl of this 10µM stock per well will result in strong inhibition (final [trichostatin A] = 500 nM)

FLUOR DE LYS Developer II (5x Concentrate, Cat. # BML-KI176)

Shortly before use, dilute 5x stock solution to 1x plus 2µM trichostatin A. For example, prepare 1ml by mixing 200µl of the 5x Concentrate, 790µl HDAC Assay Buffer II (BML-KI422) and 10µl 0.2mM trichostatin A in DMSO. Store the 1x Developer II plus trichostatin on ice until use. Do not store excess, but prepare freshly as needed.

Handle with care

FLUOR DE LYS® Deacetylated Standard (BML-KI142)

Dilute the 10mM stock in DMSO to 1µM with HDAC Assay Buffer II (BML-KI422).

½ Volume 96-well white NBS micro-plate (BML-KI571)

REACTION CONDITION EXAMPLES :

1. Designate wells for four reactions: 30 min rxn; 30 min rxn plus trichostatin A; 0 min rxn and a Standard well.
2. Add 20µl of HDAC Assay Buffer II to the 30 min rxn well and the 0 min rxn well. To the third well (30 min. plus trichostatin) add 2.5µl of 10µM trichostatin A plus 17.5µl of HDAC Assay Buffer II. Allow to equilibrate to assay temperature (37°C). (Leave Standard well empty until step 7).
3. Add 5µl of diluted HDAC11 (BML-SE560, 0.1µg/µl) to the wells for the 0 min, 30 min, and 30 min. plus trichostatin rxns.
4. To start reactions, add 25µl of the 2x Substrates (37°C) to both 30 min reaction wells. Allow reactions to run 30 min @ 37°C.
5. Add 50µl of 1x Developer II plus trichostatin A to both 30 min. reaction wells.
6. To the 0 min rxn well, add 50µl of 1x Developer II plus trichostatin A, immediately followed by 25µl of 2x substrate.
7. For the standard, mix 50µl of 1µM standard with 50µl of 1x Developer II plus trichostatin A 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.
9. Data analysis: Determine the ΔAFU (Arbitrary Fluorescence Units) for the two 30 min rxns. (AFU of 30 min rxn. (with or without trichostatin) minus AFU of the 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 ΔAFU by AFU/pmol).

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