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Data Sheet

Fluorogenic HDAC/SIRT Substrate

Catalog #: 50032

DESCRIPTION: A fluorogenic, acetylated peptide substrate for HDACs and SIRTs. 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)1-3, it is a suitable substrate for HDAC2, HDAC3, HDAC6; SIRT1, SIRT2 and SIRT3 tested so far. This substrate should be used in conjunction with HDAC or SIRT Developer, respectively.

PURITY: >90% by HPLC

MOLECULAR WEIGHT: 612.6

APPLICATIONS: Study for enzyme activity, and screening of small molecule inhibitors for drug discovery and HTS applications.

SUPPLIED AS: 5 mM solution in DMSO

QUANTITY: 100 µl

STORAGE: -20°C or -70°C. Protect from exposure to direct light. Avoid freeze/thaw cycles.

REFERENCES:

1. A. Ito *et al.* (2001) *EMBO J*. **20:** 1331.
2. N.A. Barlev *et a*l. (2001) *Mol. Cell* **8:** 1243.
3. A. Ito *et al.* (2002) *EMBO J.* **21:** 6236.

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Assay Protocol HDAC:

Materials:
Assay buffer (BPS catalog # 50031)
Assay developer (BPS catalog # 50030)
HDAC Substrate (BPS catalog # 50032)

Step 1: Adding all reaction mixture to a low binding NUNC black plate (VWR catalog number 62408-936)

1 µl of HDAC2 (0.1 µg/µl)
39 µl of HDAC assay buffer (BPS catalog # 50031)
5 µl of 1 mg/ml BSA
5 µl of 200 µM substrate (BPS catalog # 50032)

Incubate at 37 ºC for 30 min.

Step 2: Stop the reaction
Add 50 µl of HDAC assay developer (2x) (BPS catalog # 50030) and incubate the plate at room temperature for 15 min.

Step 3:
Read sample in a microtiter-plate reading fluorimeter capable of excitation at a wavelength in the range 350-380 nm and detection of emitted light in the range 440-460 nm.

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Assay Protocol SIRT:

Materials:
SIRT Assay buffer (BPS catalog #50090)
SIRT Assay developer (BPS catalog #50089)
SIRT Substrate (BPS catalog #50080)
SIRT1 (1 µg/µl) (BPS catalog #50012) or other SIRT enzyme

Step 1: Adding all reaction mixture to a low binding NUNC black plate (VWR catalog number 62408-936):
SIRT Assay Buffer, 35 µl
1 mg/ml BSA, 5 µl
200 µM SIRT substrate, 5 µl
SIRT1, 5 µl

Incubate at 37°C for 30 min.

Step 2: Stop the reaction
Add 50 µl of SIRT assay developer (undiluted) and incubate the plate at room temperature for 15 min.

Step 3:
Read samples in a microtiter-plate reading fluorimeter capable of excitation at 350-380 nm and emission at 440-460 nm. “Blank” value (no enzyme negative control) is subtracted from all other values.

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