

Data Sheet

Fluorogenic Sirtuin 5 Substrate

Catalog #: WB-1000

DESCRIPTION: A fluorogenic, succinylated peptide substrate for Sirtuin 5. This substrate should be used in conjunction with SIRT Developer, # WB-1001. Optimal excitation wavelength 350–380 nm, emission wavelength 440–460 nm.

PURITY: >90% by HPLC.

APPLICATIONS: Suitable for use with West Bioscience's Fluorogenic SIRT5 Assay Kit, # WB-1002, and to study enzyme kinetics and screen small molecule inhibitors of Sirtuin 5 for drug discovery and HTS applications.

SUPPLIED AS: DMSO solution

QUANTITY: 50 µl @ 5 mM

STORAGE: -20°C or -70°C. Avoid freeze/thaw cycles. Stable for at least 6 months from date of receipt when stored as directed. Protect from light.

REFERENCES:

1. Madsen, A.S. and Olsen, C.A., *J. Med. Chem.* 2012, **55**:5582-5590.
2. Du, J., *et al.*, *Science*, 11 November 2011, **334**:806-809.

ASSAY PROTOCOL:

Materials:

SIRT Assay Buffer (WB-1003) # 50090
SIRT5 Substrate (# WB-1000)
SIRT5 enzyme (# WB-1004) or other SIRT5-containing sample
NUNC black 96-well plate, low protein binding (VWR #62408-936).
SIRT Assay Developer (West Bioscience # WB-1001)

Step 1:

Set up 50 µl of Sirt5 reactions containing SIRT assay buffer, 400 ng SIRT5 enzyme, and 20 µM SIRT5 substrate in the 96-well plate. Incubate at 37°C for 30 min.

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Step 2:

Add 50 μ l of SIRT Assay Developer (2x) (WestBio #w60100) to each well 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. "Blank" value (no enzyme control) is subtracted from all other values.

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