Data Sheet

SIRTainty™ Class III HDAC Assay

The most flexible and reliable assay for measuring sirtuin activity

The SIRTainty™ Class III HDAC Assay utilizes a novel patent pending technology for the sensitive detection of all known sirtuin family members. Unlike conventional assays that are dependent upon a single pre-labeled fluorescently tagged substrate, the SIRTainty Class III HDAC Assay employs untagged acetylated peptide substrates (Figure 1). This approach not only enables unparalleled flexibility in your choice of sirtuin isoform and peptide substrate, but also eliminates the potential for artifacts due to the use of artificial substrates containing bulky fluorophores.

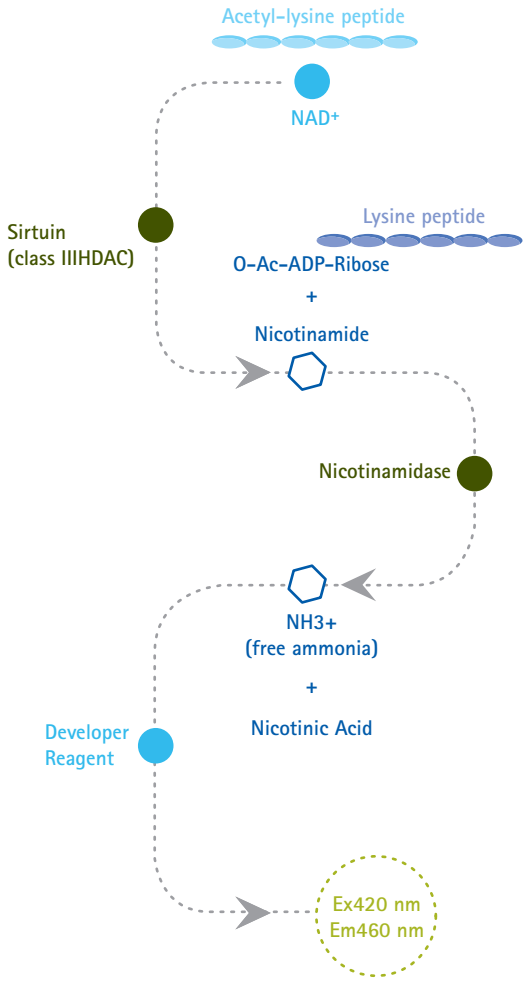

Figure 1.
Assay Format

Reliably measure and identify true modulators of sirtuin activity:

- **Sensitive Detection**
  With a limit of detection as low as 0.16U (Figure 2) of purified sirtuin, the SIRTainty assay provides almost five fold greater sensitivity than assays dependent upon a labeled substrate.
- **Unparalleled Flexibility**
  Analyze multiple sirtuin isoforms, perform inhibitor/activator assays, or characterize enzyme mechanisms using the provided peptide substrate or your own custom substrate.
- **Reliable Chemistry**
  Robust assay that is not dependent on a labeled peptide substrate thus avoiding detection artifacts due to compounds such as resveratrol.
- **Easy-to-use Format**
  Homogeneous, no wash, 96-well assay minimizes hands on time and speeds up your entire workflow.

Sensitive Detection of Activity of Multiple Sirtuin Family Members

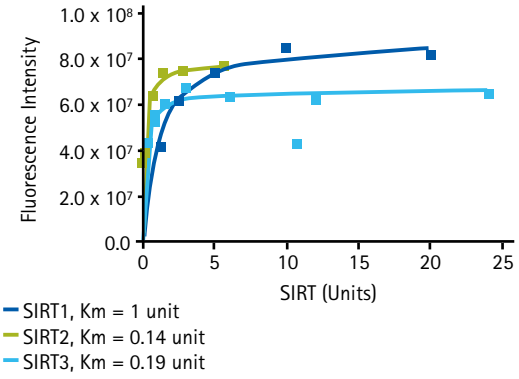

Figure 2.

Determination of Sirtuin Km values. Sirtuins 1, 2, and 3 at a range of concentrations were incubated with 25 µM acetylated peptide substrate and 0.2 mM β-NAD using the SIRTainty™ assay. The Km value was determined by non-linear curve fit of Michaelis-Menten.

Detection of Sirtuin Activity Using Variety of Substrates and Enzymes

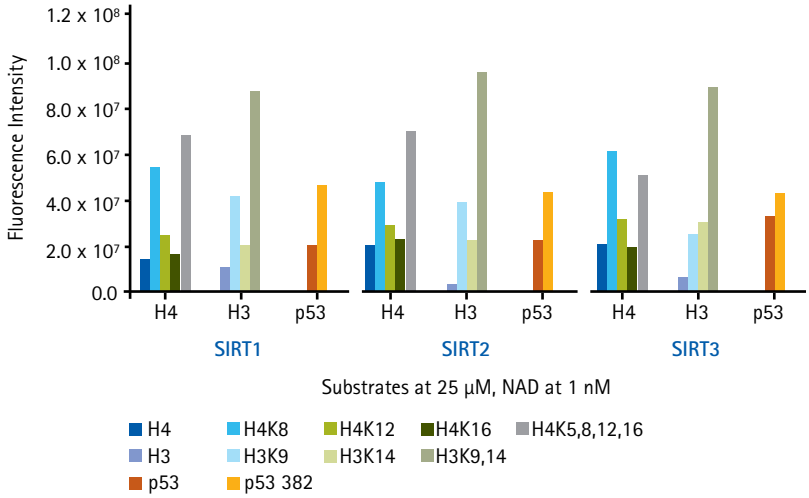

Figure 3.

Activity of Sirt1, 2, and 3 with a panel of Acetylated Peptide Substrates. Peptide substrates corresponding to p53, histone H3 and histone H4 acetylation sites were selected. Peptides were either unacetylated (H4, H3 and p53), acetylated at one site (e.g. H4K8), or acetylated at more than one site (e.g. H3K9,14). All peptides used are available from Millipore. Recombinant Sirt1 (5 Unit), Sirt2 (0.7 Unit) and Sirt3 (3 Unit) were incubated with indicated acetylated peptide substrates (25 µM) and 1mM β-NAD with the SIRTainty assay. H3K9, H3K9/14, H4K8, and H4K5/8/12/16 displayed high efficiency for deacetylation as compared to the corresponding non-acetylated peptides for all 3 enzymes tested.

Ordering Information

Description	Qty/Pk	Catalogue No.
SIRTainty Class III HDAC Assay	1 kit	17-10090
Calbiochem® Brand Related and Accessory Products		
SIRT1, GST-Fusion, Human, Recombinant, *E. coli*		524743
SIRT2, His•Tag®, Human, Recombinant, *E. coli*		524744
SIRT3, GST-Fusion, Human, Recombinant, *E. coli*		524745
Suramin, Sodium Salt		574625
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SIRT1/2 Inhibitor VII		566327
SIRT1/2 Inhibitor VIII, Salermide		566330
SIRT1 Inhibitor III		566322
SIRT1 Inhibitor IV, (S)-35		566325
SIRT2 Inhibitor, AGK2		566324
SIRT2 Inhibitor II, AK-1		566331
Sirtinol		566320
InSolution™ Sirtinol		566321

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