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Assay Report

Histone Deacetylase (HDAC) Inhibitor Assays Enzymatic Study of Compounds from Client



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Client_HDAC_Year Month Year

HDAC Inhibitor Assays

Study Sponsor: Client

Attention:

Address:

Study Director: Henry Zhu, Ph.D.

Testing Facility: BPS Bioscience Inc.
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Study Period:

Report Version: 1

Report Date: Month Day, Year



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<h2>Study Director</h2>

Scientist

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Purpose of the Study

The purpose of the study is to determine the effects of compounds from Client on the activities of recombinant HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC6, HDAC7, HDAC8, HDAC9, HDAC10, HDAC11, Sirtuin1, Sirtuin2, Sirtuin3, and Sirtuin5 using an *in vitro* enzymatic assay.



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2. Materials and Methods

2.1 Materials

SAHA is purchased from Cayman Chemicals (Ann Arbor, MI, Catalog Number 10009929).

Nicotinamide is purchased from Sigma-Aldrich (St. Louis, MO, Catalog number 72340).

TSA is purchased from Selleck (Houston, TX, Catalog number S1045).

HDAC Assay Buffer (BPS catalog number 50031)

HDAC Assay Developer (BPS catalog number 50030)

SIRT Assay Developer (BPS catalog number 50089)

HDAC Substrate 1 (BPS catalog number 50032)

HDAC Substrate 3 (BPS catalog number 50037)

HDAC Class 2a Substrate 1 (BPS catalog number 50040)

Sirtuin 5 Substrate (BPS catalog number 50126)

2.2 Compounds

The test compounds are supplied by AMSBIO, LLC.

Compound I.D.	Compound Supplied	Stock Concentration	Dissolving Solvent	Test Range (μ M)	Intermediate Dilution
SAHA*	Solid	10mM	DMSO	0.0003 - 10	10 % DMSO in HDAC Assay Buffer
TSA*	Solid	10mM	DMSO	0.003 - 100	10 % DMSO in HDAC Assay Buffer
Nicotinamide*	Solid	100mM	H ₂ O	0.03 - 1000	10 % DMSO in HDAC Assay Buffer

*Reference Compound



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2.3 Experimental Conditions

2.3.1 Enzymes and Substrates

Assay	Catalog #	Enzyme Lot #	Enzyme Used (ng) / Reaction	Substrate
HDAC1	50051	150528-B	7.2	10 μ M HDAC Substrate 3
HDAC2	50002	151125-G	7.5	10 μ M HDAC Substrate 3
HDAC3/NCOR2	50003	130819	3.4	10 μ M HDAC Substrate 3
HDAC4	50004	130115	0.3	2 μ M HDAC Substrate Class 2a
HDAC5	50005	140211	45	2 μ M HDAC Substrate Class 2a
HDAC6	50006	150603-A	10	10 μ M HDAC Substrate 3
HDAC7	50007	150629-5	1.6	2 μ M HDAC Substrate Class 2a
HDAC8	50008	150714	22	2 μ M HDAC Substrate Class 2a
HDAC9	50009	130502-66	4.3	2 μ M HDAC Substrate Class 2a
HDAC10	50060	150709-1B	500	10 μ M HDAC Substrate 3
HDAC11	50021	141104	50	2 μ M HDAC Substrate Class 2a
SIRT1	50012	140211-1	700	10 μ M HDAC Substrate 1
SIRT2	50013	150924-B	4000	10 μ M HDAC Substrate 1
SIRT3	50014	150903-B	1600	10 μ M HDAC Substrate 1
SIRT5	50016	140813-1	300	10 μ M SIRT5 Substrate



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2.3.2 Assay Conditions

All of the compounds are dissolved in DMSO. The serial dilution of the compounds was first performed in 100% DMSO with the highest concentration at 1mM. Each intermediate compound dilution (in 100% DMSO) will then get directly diluted 10x fold into assay buffer for an intermediate dilution of 10% DMSO in HDAC assay buffer and 5µl of the dilution was added to a 50µl reaction so that the final concentration of DMSO is 1% in all of reactions.

The enzymatic reactions for the HDAC enzymes were conducted in duplicate at 37°C for 30 minutes in a 50µl mixture containing HDAC assay buffer, 5µg BSA, an HDAC substrate (see 2.3.1), a HDAC enzyme (see 2.3.1) and a test compound (see 2.2).

The enzymatic reactions for the SIRT enzymes were conducted in duplicate at 37°C for 30 minutes in a 50µl mixture containing HDAC assay buffer, 5µg BSA, 0.5mM NAD⁺ an HDAC substrate (see 2.3.1), a SIRT enzyme (see 2.3.1) and a test compound (see 2.2).

After enzymatic reactions, 50µl of 2 x HDAC Developer was added to each well for the HDAC enzymes and the plate was incubated at room temperature for an additional 15 minutes.

After enzymatic reactions, 50µl of 2 x SIRT Developer was added to each well for the SIRT enzymes and the plate was incubated at room temperature for an additional 15 minutes.

Fluorescence intensity was measured at an excitation of 360 nm and an emission of 460 nm using a Biotek Synergy microplate reader.



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2.3.3 Data Analysis

HDAC activity assays were performed in duplicates at each concentration. The fluorescent intensity data were analyzed using the computer software, Graphpad Prism. In the absence of the compound, the fluorescent intensity (F_t) in each data set was defined as 100% activity. In the absence of HDAC, the fluorescent intensity (F_b) in each data set was defined as 0% activity. The percent activity in the presence of each compound was calculated according to the following equation: %activity = $(F - F_b) / (F_t - F_b)$, where F= the fluorescent intensity in the presence of the compound.

The values of % activity versus a series of compound concentrations were then plotted using non-linear regression analysis of Sigmoidal dose-response curve generated with the equation $Y = B + (T - B) / (1 + 10^{((\text{LogEC50} - X) \times \text{Hill Slope})})$, where Y=percent activity, B=minimum percent activity, T=maximum percent activity, X= logarithm of compound and Hill Slope=slope factor or Hill coefficient. The IC50 value was determined by the concentration causing a half-maximal percent activity.

3. Assay Results

3.1. Summary of the Inhibitory Effects of the Compounds on HDAC and Sirtuin Activities

The IC₅₀ of the compound against HDACSs and SIRTs are summarized on Table 3.1. If the IC₅₀ is higher than XμM, the percentage inhibition of the compound at XμM is calculated.

Table 3.1 Inhibitory Effects of the Compound on HDAC and SIRT Activities (IC₅₀)

Enzymes	IC ₅₀ (μM) or Percentage Inhibition		
	SAHA	TSA	Nicotinamide
HDAC1	0.031		
HDAC2	0.067		
HDAC3/NCOR2	0.028		
HDAC4		3.5	
HDAC5		1.8	
HDAC6	0.011		
HDAC7		1.4	
HDAC8		0.63	
HDAC9		4.4	
HDAC10	0.082		
HDAC11		12.1	
SIRT1			177
SIRT2			41
SIRT3			21
SIRT5			12

3.2. Results of the Effects of the Compounds on Individual HDAC Activity

3.2.1. HDAC1

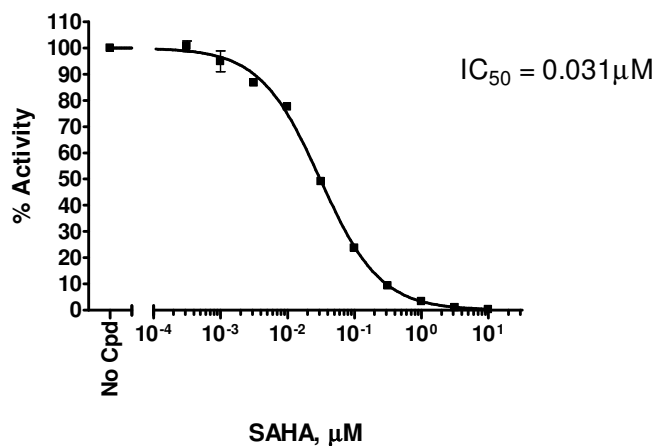
3.2.1.1. SAHA

Table 3.2.1.1. Data for the Effect of SAHA on HDAC1 Activity

SAHA [μ M]	HDAC1 Activity (Fluorescence count)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	49943	48920	101	99
0.0003	48766	50712	99	103
0.001	48883	45138	99	91
0.003	43826	42434	88	85
0.01	38801	38795	77	77
0.03	25986	24661	50	48
0.1	13125	13600	23	24
0.3	6450	6685	9	9
1	3696	3768	3	3
3	2700	2627	1	1
10	2252	2337	0	0
Background	2142	2262	0	0

HDAC1 Activity

Substrate Conc.=10 μ M Substrate 3 (50037)



3.2.2. HDAC2

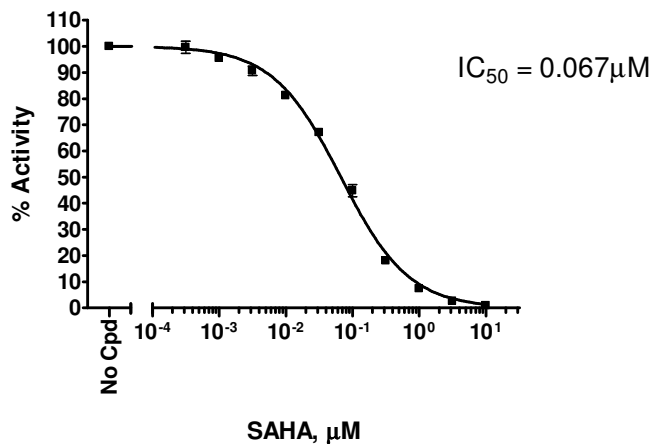
3.2.2.1. SAHA

Table 3.2.2.1. Data for the Effect of SAHA on HDAC2 Activity

SAHA [μ M]	HDAC2 Activity (Fluorescence count)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	12929	13140	99	101
0.0003	12716	13251	97	102
0.001	12483	12524	95	96
0.003	12147	11741	92	89
0.01	10759	10929	80	82
0.03	9269	9147	68	67
0.1	6346	6887	42	47
0.3	3556	3442	18	18
1	2265	2269	7	7
3	1795	1630	3	2
10	1472	1547	1	1
Background	1399	1412	0	0

HDAC2 Activity

Substrate Conc.=10 μ M Substrate 3 (50037)



3.2.3. HDAC3/NCOR2

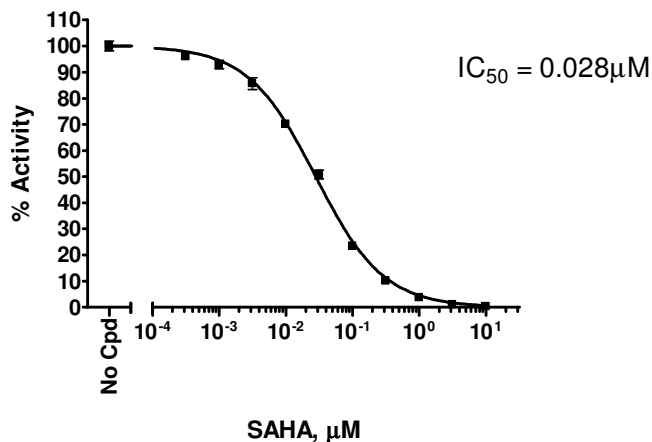
3.2.3.1. SAHA

Table 3.2.3.1. Data for the Effect of SAHA on HDAC3/NCOR2 Activity

SAHA [μ M]	HDAC3/NCOR2 Activity (Fluorescence count)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	32041	33155	98	102
0.0003	31061	31619	95	97
0.001	29867	30904	91	95
0.003	27402	28819	83	88
0.01	22848	23676	69	71
0.03	17780	16735	53	49
0.1	8714	8680	23	23
0.3	4601	4439	10	10
1	2711	2384	4	3
3	1715	1654	1	1
10	1510	1446	0	0
Background	1386	1401	0	0

HDAC3/NCOR2 Activity

Substrate Conc.=10 μ M Substrate 3 (50037)



3.2.4. HDAC4

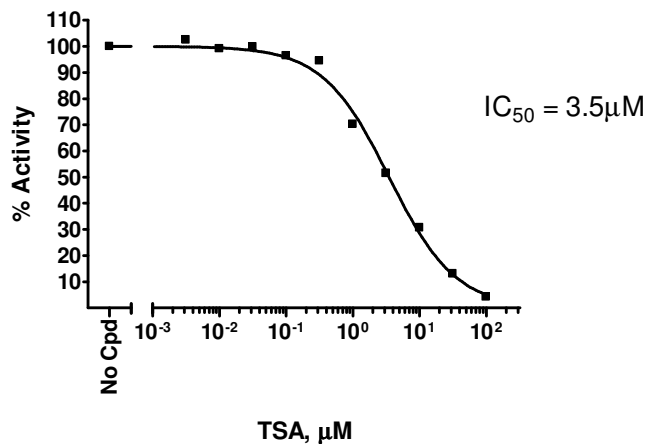
3.2.4.1. TSA

Table 3.2.4.1. Data for the Effect of TSA on HDAC4 Activity

TSA [μ M]	HDAC4 Activity (Fluorescence count)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	28908	28440	100	99
0.003	28997	29673	100	100
0.01	28258	28572	98	100
0.03	28517	28734	99	100
0.1	27643	27771	96	97
0.3	27305	27070	95	94
1	20730	20553	71	70
3	15682	15467	52	51
10	10005	9888	31	30
30	5346	5059	14	13
100	2791	2805	4	4
Background	1673	1671	0	0

HDAC4 Activity

Substrate Conc.=2 μ M Class 2a Substrate 1 (50040)



3.2.5. HDAC5

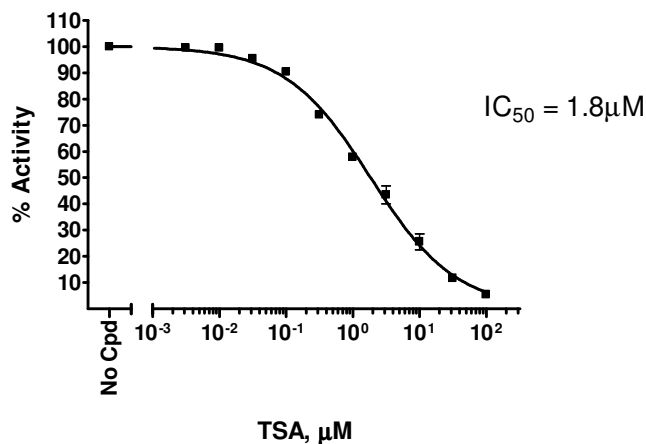
3.2.5.1. TSA

Table 3.2.5.1. Data for the Effect of TSA on HDAC5 Activity

TSA [μ M]	HDAC5 Activity (Fluorescence count)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	12690	12821	99	101
0.003	12656	12711	99	100
0.01	12707	12658	100	99
0.03	12279	12090	96	95
0.1	11632	11447	91	89
0.3	9437	9577	73	74
1	7551	7448	58	57
3	6148	5288	47	40
10	3116	3861	23	29
30	1589	1929	10	13
100	995	963	5	5
Background	310	318	0	0

HDAC5 Activity

Substrate Conc.=2 μ M Class 2a Substrate 1 (50040)



3.2.6. HDAC6

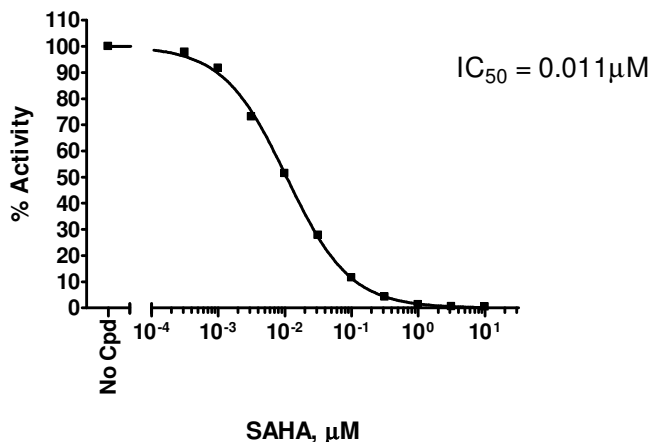
3.2.6.1. SAHA

Table 3.2.6.1. Data for the Effect of SAHA on HDAC6 Activity

SAHA [μ M]	HDAC6 Activity (Fluorescence count)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	32065	32780	99	101
0.0003	31720	31707	98	98
0.001	30178	29579	93	91
0.003	24529	24021	74	72
0.01	17902	17567	52	51
0.03	10504	10722	27	28
0.1	5661	5733	11	12
0.3	3504	3432	4	4
1	2582	2580	1	1
3	2323	2420	0	1
10	2447	2255	1	0
Background	2297	2140	0	0

HDAC6 Activity

Substrate Conc.=10 μ M Substrate 3 (50037)



3.2.7. HDAC7

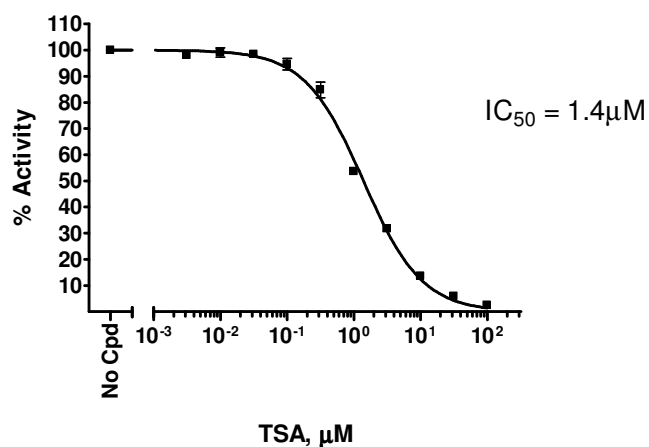
3.2.7.1. TSA

Table 3.2.7.1. Data for the Effect of TSA on HDAC7 Activity

TSA [μ M]	HDAC7 Activity (Fluorescence count)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	35177	36031	99	101
0.003	34792	35024	98	98
0.01	34704	35871	97	101
0.03	34760	35282	98	99
0.1	34534	33018	97	92
0.3	31473	29447	88	82
1	19804	20064	53	54
3	12110	13000	30	33
10	6524	6382	14	13
30	3839	3847	6	6
100	2666	2724	2	3
Background	1868	1831	0	0

HDAC7 Activity

Substrate Conc.=2 μ M Class 2a Substrate 1 (50040)



3.2.8. HDAC8

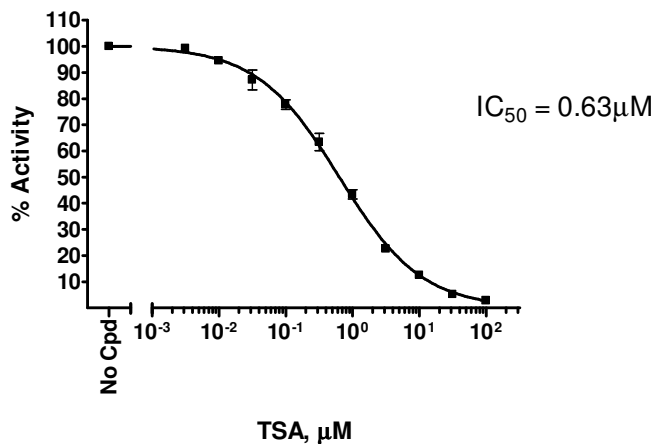
3.2.8.1. TSA

Table 3.2.8.1. Data for the Effect of TSA on HDAC8 Activity

TSA [μ M]	HDAC8 Activity (Fluorescence count)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	7088	6896	101	99
0.003	6860	7016	98	100
0.01	6656	6596	95	94
0.03	5885	6388	83	91
0.1	5628	5385	80	76
0.3	4774	4336	67	60
1	3110	3348	42	45
3	1815	1850	22	23
10	1150	1182	12	13
30	722	634	6	4
100	518	520	3	3
Background	343	326	0	0

HDAC8 Activity

Substrate Conc.=2 μ M Class 2a Substrate 1 (50040)



3.2.9. HDAC9

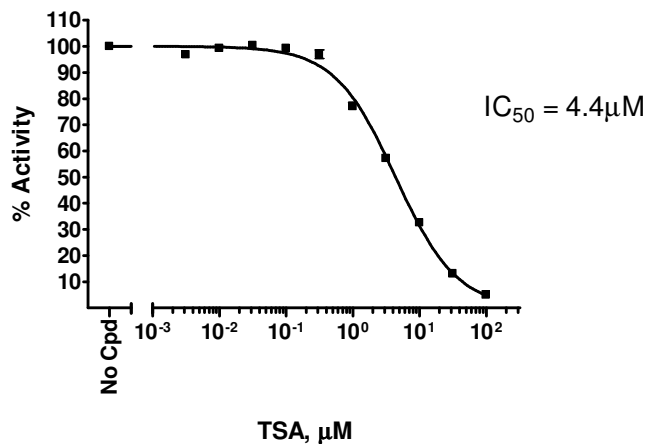
3.2.9.1. TSA

Table 3.2.9.1. Data for the Effect of TSA on HDAC9 Activity

TSA [μ M]	HDAC9 Activity (Fluorescence count)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	17614	17847	99	100
0.003	17036	17413	96	98
0.01	17629	17586	99	99
0.03	17916	17633	100	99
0.1	17608	17615	99	99
0.3	17515	16998	99	95
1	14381	13890	79	75
3	11189	10880	58	56
10	7293	7113	33	32
30	4164	4128	13	13
100	2882	2893	5	5
Background	2105	2105	0	0

HDAC9 Activity

Substrate Conc.=2 μ M Class 2a Substrate 1 (50040)



3.2.10. HDAC10

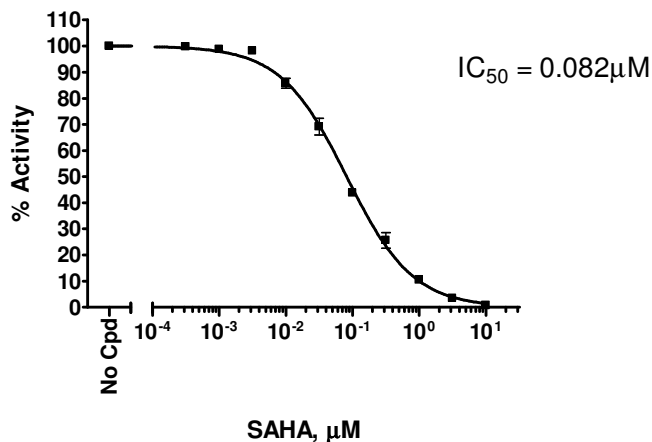
3.2.10.1. SAHA

Table 3.2.10.1. Data for the Effect of SAHA on HDAC10 Activity

SAHA [μ M]	HDAC10 Activity (Fluorescence count)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	16926	16469	102	98
0.0003	16428	16879	98	101
0.001	16619	16402	99	98
0.003	16380	16465	98	98
0.01	14297	14847	84	88
0.03	12574	11632	72	66
0.1	8261	8419	43	44
0.3	6084	5186	29	23
1	3301	3477	10	11
3	2326	2369	3	4
10	1914	1945	1	1
Background	1827	1833	0	0

HDAC10 Activity

Substrate Conc.=10 μ M Substrate 3 (50037)



3.2.11. HDAC11

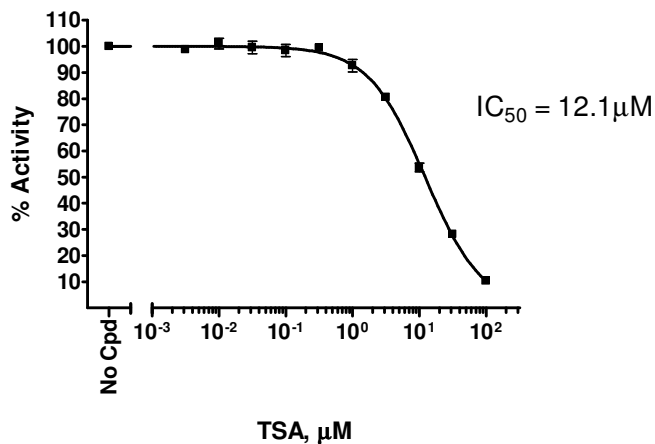
3.2.11.1. TSA

Table 3.2.11.1. Data for the Effect of TSA on HDAC11 Activity

TSA [μ M]	HDAC11 Activity (Fluorescence count)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	17242	16777	102	98
0.003	16610	16990	97	100
0.01	17490	16849	103	99
0.03	16565	17306	97	102
0.1	17121	16403	101	96
0.3	16833	16982	99	100
1	15494	16247	90	95
3	13858	14173	80	82
10	10140	9626	55	52
30	6042	5866	29	28
100	3157	3259	10	11
Background	1606	1621	0	0

HDAC11 Activity

Substrate Conc.=2 μ M Class 2a Substrate 1 (50040)



3.2.12. SIRT1

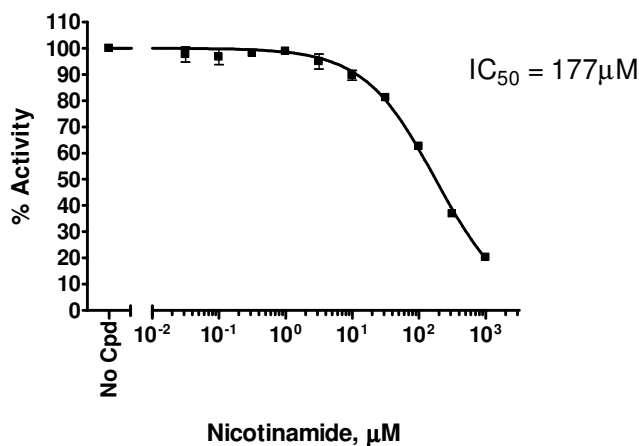
3.2.12.1. Nicotinamide

Table 3.2.12.1. Data for the Effect of Nicotinamide on SIRT1 Activity

Nicotinamide [μ M]	SIRT1 Activity (Fluorescence count)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	50191	51094	99	101
0.03	48064	50890	95	101
0.1	47579	50422	94	100
0.3	48979	50489	97	100
1	50474	49656	100	98
3	46746	49554	92	98
10	44641	46489	88	92
30	41033	41769	80	82
100	32032	32486	62	63
300	19232	19960	36	38
1000	11330	11468	20	20
Background	1493	1469	0	0

SIRT1 Activity

Substrate Conc.=10 μ M Substrate 1 (50032)



3.2.13. SIRT2

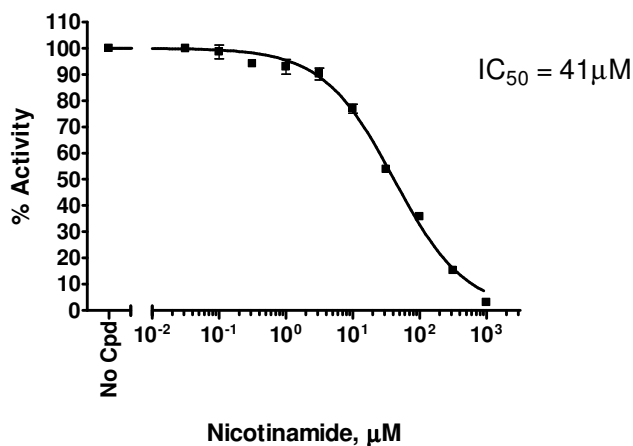
3.2.13.1. Nicotinamide

Table 3.2.13.1. Data for the Effect of Nicotinamide on SIRT2 Activity

Nicotinamide [μ M]	SIRT2 Activity (Fluorescence count)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	38736	38620	100	100
0.03	38240	39051	99	101
0.1	37320	39091	96	101
0.3	36204	37213	93	96
1	35365	37277	90	96
3	34661	36142	88	92
10	30444	31571	75	79
30	22931	23694	53	55
100	17150	17449	35	36
300	10232	10735	14	16
1000	6461	6395	3	3
Background	5429	5470	0	0

SIRT2 Activity

Substrate Conc.=10 μ M Substrate 1 (50032)



3.2.14. SIRT3

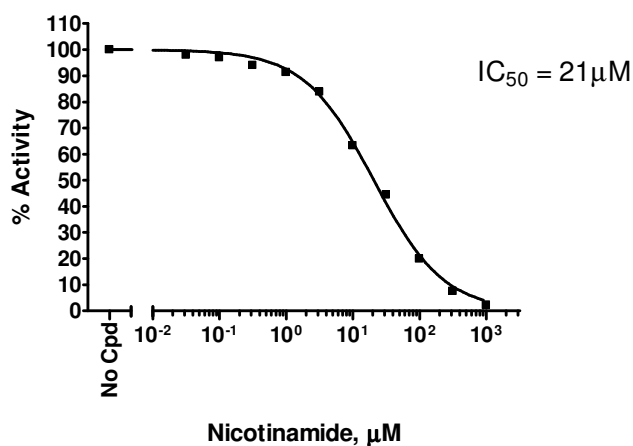
3.2.14.1. Nicotinamide

Table 3.2.14.1. Data for the Effect of Nicotinamide on SIRT3 Activity

Nicotinamide [μ M]	SIRT3 Activity (Fluorescence count)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	22838	23173	99	101
0.03	22578	22541	98	98
0.1	22299	22395	97	97
0.3	21702	21698	94	94
1	20926	21289	90	92
3	19442	19545	84	84
10	15159	14896	64	63
30	11051	10804	45	44
100	5526	5716	19	20
300	2966	2936	8	7
1000	1803	1781	2	2
Background	1336	1309	0	0

SIRT3 Activity

Substrate Conc.=10 μ M Substrate 1 (50032)



3.2.15. SIRT5

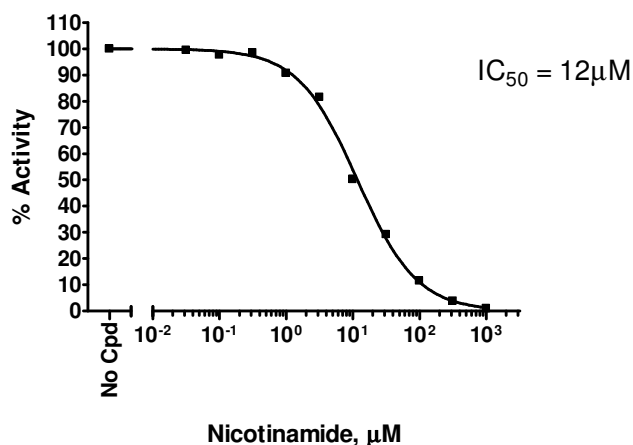
3.2.15.1. Nicotinamide

Table 3.2.15.1. Data for the Effect of Nicotinamide on SIRT5 Activity

Nicotinamide [μ M]	SIRT5 Activity (Fluorescence count)		% Activity	
	Repeat1	Repeat2	Repeat1	Repeat2
No Compound	13888	13975	100	100
0.03	14013	13682	101	98
0.1	13582	13651	97	98
0.3	13672	13774	98	99
1	12757	12614	91	90
3	11489	11445	82	81
10	7448	7156	51	49
30	4509	4480	29	29
100	2183	2084	12	11
300	1130	1092	4	4
1000	734	716	1	1
Background	612	613	0	0

SIRT5 Activity

Substrate Conc.=10 μ M Substrate 5 (50126)





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4. Quality Assurance Statement

I certify that the results presented in this report were generated using the materials and methods mentioned and that these results reflect the Raw Data.

Henry Zhu, Ph.D.
President

Date