

## ab287850 – Sirtuin Activity Assay Kit

For the detection of incorporated BrdU using a mouse anti-BrdU antibody.  
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab287850>

### Storage and Stability

Store unopened kit at -80°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

### Materials Supplied

Item	Quantity	Storage Condition
1M DTT	0.4 mL	-80°C
AFC Standard (in DMSO) (1 mM)	0.1 mL	-80°C
Developer	1 mL	-80°C
Homogenization buffer	100 mL	-80°C
NAD	1 vial	-80°C
Positive Control	20 µL	-80°C
Sirtuin Assay buffer	25 mL	-80°C
Substrate (in DMSO)	0.2 mL	-80°C
Tichostatin A (in DMSO)	50 µL	-80°C

### Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well plate with flat bottom. White plate is preferred for this assay.
- Fluorescence microplate reader
- Protease Inhibitor Cocktail

### Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

**Sirtuin Assay Buffer:** Store at 4°C or -20°C. Warm to 37°C and add DTT to final concentration of 2 mM just before use. Make fresh as needed.

**Homogenization Buffer:** Thaw at room temperature and keep on ice while in use.

**Substrate:** Aliquot and Store at -20°C. Avoid repeated freeze/thaw. Use fresh tip each time.

**NAD:** Reconstitute with 220 µL deionized water. Aliquot and store at -80°C after reconstituting. Avoid repeated freeze/thaw.

**Positive Control:** Store at -80°C. Avoid repeated freeze/thaw.

**Trichostatin A:** Store at -20°C. Use fresh tip each time.

**Developer:** Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use.

### Sirtuin Activity Assay protocol

#### Sample Preparation:

1. Add DTT to Homogenization Buffer to a final concentration of 2 mM. Make fresh as needed.
2. Rinse tissue and transfer ~100 mg of fresh or frozen tissue (stored at -80°C) to a prechilled tube.
3. Add 600 µL cold Homogenization Buffer containing protease inhibitor cocktail (not provided) and thoroughly homogenize tissue on ice.
4. Transfer the tissue homogenate to a cold microfuge tube. To prepare cell extract, add 150-300 µL cold Homogenization Buffer containing protease inhibitor cocktail (not provided) to 1-5 x 10<sup>6</sup> fresh or frozen cells and homogenize cells on ice.
5. Transfer the cell homogenate including cell debris to a cold microfuge tube and agitate on a rotary shaker at 4°C for 15 min. Centrifuge the tissue or cell homogenate at 16,000 X g for 20 min. at 4°C.
6. Transfer the clarified supernatant to a fresh pre-chilled tube and keep on ice. Use lysates immediately to assay for Sirtuin Activity.

**Δ Note:** Lysates can be aliquoted and snap frozen in liquid nitrogen before storing at -80°C. Avoid freeze/thaw.

#### AFC Standard:

1. Dilute AFC Standard to 10 µM by adding 10 µL of 1 mM AFC Standard to 990 µL of Sirtuin Assay Buffer (with DTT). Add 0, 20, 40, 60, 80, and 100 µL of diluted 10 µM AFC Standard into individual wells in a 96-well white plate and adjust the volume to 100 µL/well with Sirtuin Assay Buffer (with DTT) to generate 0, 200, 400, 600, 800, and 1000 pmol/well of AFC Standard respectively. Mix well.

#### Sirtuin Activity Assay:

1. Add 2-50 µL of cell/tissue homogenate/nuclear extract/mitochondria or purified protein into desired wells in a 96-well plate.
2. For positive control, add 2 µL of Positive Control in desired well.
3. Make up the volume of samples and Positive Control to 50 µL/well with Sirtuin Assay Buffer (with DTT).

#### Δ Notes:

- a) For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
- b) For samples having HDAC activity, it is recommended using two wells for each sample, one with (1µL) and the other without Trichostatin A. You can measure the Sirtuin Activity and HDAC + Sirtuin Activity respectively.
- c) Use heat inactivated sample as sample background control. For Positive Control Background, use Sirtuin Assay Buffer (with DTT) as background control. Make up the volume to 50 µL/well with Sirtuin Assay Buffer (with DTT).

#### Reaction Mix:

1. Prepare enough reagents for the number of assays to be performed. Make 40 µL Reaction Mix for each well containing:

Item	Reaction Mix
Sirtuin Assay buffer (with DTT)	36 µL
Substrate	2 µL
NAD	2 µL

2. Add 40 µL of Reaction Mix into each Sample, Positive Control and Background Control wells. Mix well. Incubate at 37°C for 30-60 min.
3. After incubation, add 10 µL of Developer to each well except Standards and mix the contents. Incubate for 10-15 min. at 37°C

## Measurement

Measure fluorescence (Ex/Em = 400/505 nm) in end point mode.

## Calculations

1. Subtract 0 Standard reading from all Standard readings and plot the AFC Standard Curve.
2. Subtract sample background control reading from Sample reading. Apply the corrected sample reading to the AFC Standard Curve to get B pmol of AFC generated by Sirtuin activity in the sample wells.

$$\text{Sample Sirtuin/HDAC activity} = B/TS = \text{pmol/min}/\mu\text{g} = \mu\text{U}/\mu\text{g}$$

**Where:** **B** = AFC amount in the sample well from Standard Curve (pmol)

**T** = reaction time in min. (30-60 min.)

**S** = Sample amount ( $\mu\text{g}$ )

3. Sample Sirtuin Activity can also be expressed as mU/mg (nmol/min. deacetylated peptide generated per mg of protein).
4. Unit Definition: One unit of Sirtuin activity is the amount of enzyme that hydrolyzes the substrate to yield 1.0  $\mu\text{mol}$  of AFC/min. at 37°C.

## Technical Support

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