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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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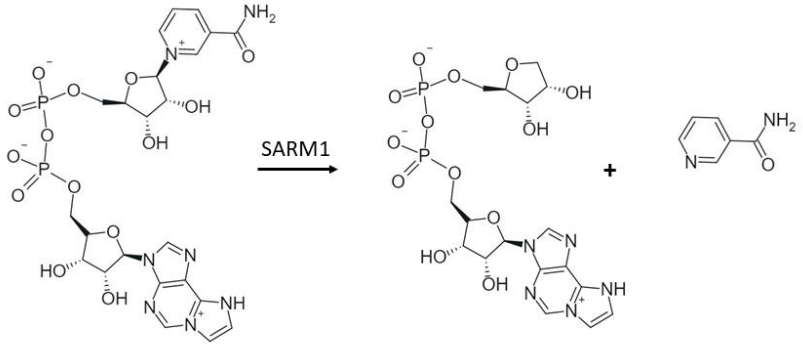
Description

The SARM1 Fluorogenic Assay Kit (Hydrolase Activity) is designed to measure NAD+ cleavage activity for screening and profiling applications. The SARM1 assay kit comes in a convenient 96-well format, with recombinant human SARM1 enzyme, its substrate N6-etheno-NAD (ε-NAD), and SARM1 assay buffer for 96 enzyme reactions. In addition, the kit includes a SARM1 inhibitor (DSRM-3716) for use as a control inhibitor.



Assay principle: Our fluorogenic assay monitors the hydrolase activity of SARM1 (hydrolysis of ENAD to form EADPR + nicotinamide). Substrate Etheno-NAD is not fluorescent due to internal quenching. However, the release of nicotinamide by SARM1 relieves quenching, which leads to an increase in fluorescence signal directly proportional to the enzymatic activity. Thus, titrating SARM1 increases the fluorescence signal in a dose dependent manner. Conversely, a SARM1 inhibitor such as DSRM-3716 reduces the fluorescence signal in a dose dependent manner.

Background

SARM1 (Sterile alpha and TIR motif containing 1) is a member of the Toll/Interleukin receptor-1 (TIR1) family of enzymes. It functions as an ADP-ribosyl cyclase and nicotinamide adenine dinucleotide (NAD) glycohydrolase. SARM1-TIR domains have intrinsic NADase activity, cleaving NAD+ into ADP Ribose (ADPR), cyclic ADPR, and Nicotinamide. Often associated with mitochondria, the protein functions as a sensor of metabolic stress. It is highly expressed in neurons, where it causes the depletion of axonal NAD+ which triggers pathological axon loss.

SARM1 functions downstream of NMNAT2 (nicotinamide nucleotide adenylyltransferase 2) to promote the active process of injury-induced neuronal degeneration known as Wallerian degeneration. Constitutive NADase activity resulting from mutation in the human SARM1 gene has been observed in neurodegenerative disease amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease). Alternatively, loss of SARM1 activity protects neurons in models of brain injury or drug-induced neuron damage. Therefore, inhibition of SARM1 NAD+ cleavage activity may potentially reduce axonal degeneration.

Applications

- Study enzyme kinetics or screen small molecular inhibitors for drug discovery and high throughput applications.

Supplied Materials

Catalog #	Name	Amount	Storage
100069	SARM1 (Recombinant Human)*	30 µg	-80°C
	4x SARM1 hydrolase buffer	3 ml	-20°C
	SARM1 substrate (ε-NAD, 12 mM)	50 µl	-20°C
	DSRM-3716 (100 mM in DMSO)	25 µl	-20°C
79685	Black 96-well plate	1	Room Temp

**The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- Adjustable micro-pipettor and sterile tips
- Fluorescent microplate reader
- Rotating or rocker platform

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

All samples and controls should be tested in duplicate. We recommend preincubating the enzyme with inhibitor.

1. Thaw **4x SARM1 hydrolase buffer** on ice.

2. Prepare **1x SARM1 hydrolase buffer** by diluting **4x SARM1 hydrolase buffer** with water. Dilute only enough buffer required for the assay. Store the remaining **4x SARM1 hydrolase buffer** at -20°C in single-use aliquots. For 96 reactions, prepare 12 ml of **1x SARM1 hydrolase buffer** by mixing 3 ml of **4x SARM1 hydrolase buffer** with 9 ml of water.

3. Add 30 µl of **1X SARM1 hydrolase buffer** to all wells.

4. Prepare the test compound by making a 100x solution in DMSO. Dilute 1: 10 with **1x SARM1 hydrolase buffer** to make a 10x solution of 10% DMSO. Add 5 µl of diluted test compound to each well labeled as "Test Inhibitor".

5. For the wells labeled “Positive Control" and “Blank”, prepare a “Diluent Solution” that contains the same concentration of diluent as the test inhibitor in 1x Kinase assay buffer (for example 10% DMSO in 1x Kinase assay buffer if the inhibitor was dissolved in DMSO), but does not contain inhibitor. Add 5 µl of the diluent solution.

5. To the wells designated as "Blank", add 10 µl of **1x SARM1 hydrolase buffer**.

6. Thaw **Recombinant Human SARM1** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Calculate the amount of **SARM1** required for the assay and dilute enzyme to 30 ng/µl with **1x SARM1 assay buffer** (the final amount of SARM1 will be 300 ng/well). Aliquot remaining **Recombinant Human SARM1** enzyme into single-use aliquots. Store remaining undiluted enzyme in aliquots at -80°C.
   Note: SARM1 enzyme is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

7. Add 10 µl of diluted **Recombinant Human SARM1** enzyme to the wells designated “Positive Control”, and "Test Inhibitors. Cover the plate and incubate 30 to 60 mins at room temperature with slow shaking.

Component	Blank	Positive Control	Test Inhibitor
1x SARM1 hydrolase buffer	40 µl	30 µl	30 µl
Test Inhibitor	-	-	5 µl
Diluent solution (no inhibitor)	5 µl	5 µl	-
Recombinant Human SARM1 (30 ng/µl)	-	10 µl	10 µl
Total	45 µl	45 µl	45 µl

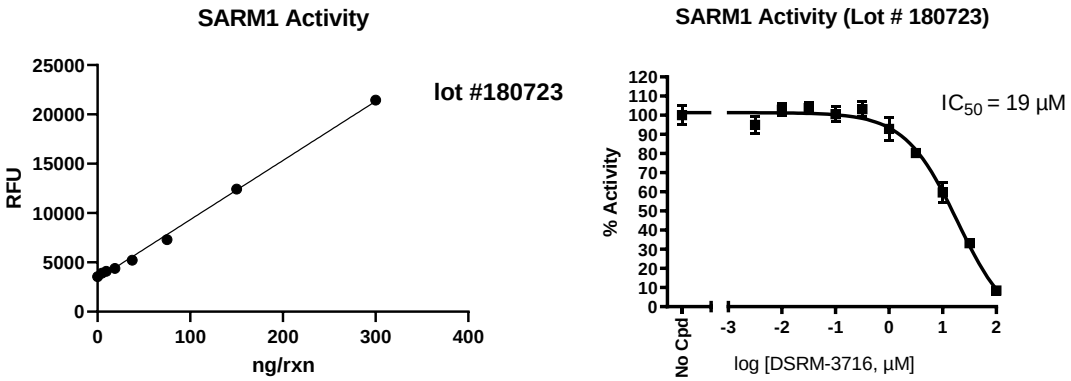
8. Meanwhile, dilute **ε-NAD** 12-fold with **1x SARM1 hydrolase buffer**. Dilute only the amount required for the assay. Store remaining **ε-NAD** at -20°C in single use aliquots. Discard any unused diluted **ε-NAD** after use.

9. After the 30- or 60-minutes pre-incubation, initiate the reaction by adding 5 µl of diluted **ε-NAD (1 mM)** to each well. This brings the final; reaction volume to 50 µl.

10. Cover the plate with aluminum foil, and incubate for 90 to 120 mins with slow shaking at room temperature.

11. After incubation, measure the plate using a fluorescence plate reader capable of excitation at 300 nm and detection of emitted light at 410 nm. The “Blank” value is subtracted from all other values.

Example Results



SARM1 activity (left) and inhibition by DSRM-3716 (right) measured using the **SARM1 Inhibitor Screening Assay Kit (Hydrolase Activity)**, BPS Bioscience #78217. Fluorescence was measured using a Bio-Tek microplate reader. Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

General Considerations

"Blank" Control: The "Blank" control is important to determine the background luminescence in the assay. We recommend doing these in duplicate.

"Positive Control":

The "Positive Control" is the maximum signal determined upon the addition of diluent solution (for example 1% DMSO in 1x CD38 Assay Buffer) in the absence of inhibitor.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
SARM1, FLAG-Tag Recombinant	100069	100 µg
NMNAT1, His-Tag Recombinant	71090	100 µg
NAD+, Biotin-Labeled	80610	100 µl
MCL-1 TR-FRET Assay Kit	79506	384 reactions
MNK1 Kinase Assay Kit	78032	96 reactions
NMNAT2, His-Tag Recombinant	100198	100 µg
NMNAT1 Assay Kit	79642	96 reactions