Description

The SARM1 Fluorogenic Assay Kit (Hydrolase Activity) is designed to measure NAD^+ cleavage activity in screening and profiling applications. The SARM1 assay kit comes in a convenient 384-well format, with enough recombinant human SARM1 enzyme (amino acids 28-724), its substrate N6-etheno-NAD (e-NAD), and SARM1 assay buffer for 384 enzyme reactions. In addition, the kit includes a SARM1 inhibitor (DSRM-3716) for use as a control inhibitor.

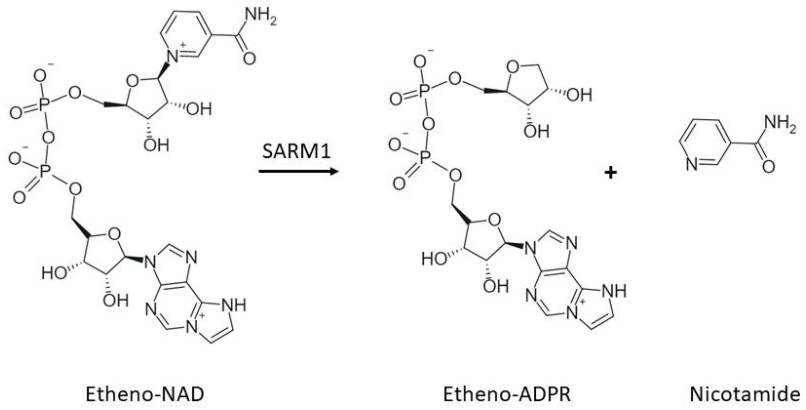

Figure 1: Assay principle.

Hydrolase activity of SARM1 is measured by following the hydrolysis of Etheno-NAD to form Etheno-ADPR + nicotinamide. Etheno-NAD is not fluorescent due to internal quenching. Upon hydrolysis by SARM1, nicotinamide is released, leading to an increase in fluorescence signal directly proportional to the enzymatic activity.

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^+ and 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 molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
100069	SARM1, FLAG-Tag Recombinant*	2 x 30 µg	-80°C
	4x SARM1 Hydrolase Buffer	2 x 3 ml	-20°C
	SARM1 Substrate (ε-NAD, 12 mM)	2 x 50 µl	-20°C
	DSRM-3716 (100 mM in DMSO)	2 x 25 µl	-20°C
79961	Black 384-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 able to excite at 300 nm and detection of emitted light at 410 nm.
- 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.
- The assay should include “Blank”, “Positive Control”, “Negative Control” and “Test inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using DSRM-3716 as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1 x, 1 x and 10 x the IC_{50} value shown in the validation data below.

1. Thaw **4x SARM1 Hydrolase Buffer** on ice.

2. Prepare **1x SARM1 Hydrolase Buffer** by diluting **4x SARM1 Hydrolase Buffer 4-fold** with distilled water.

   Note: Dilute only enough buffer required for the assay. Store the remaining 4x SARM1 Hydrolase Buffer at -20°C in single-use aliquots. For 384 reactions, prepare 12 ml of 1x SARM1 Hydrolase Buffer will be enough.

3. Add 15 µl of 1X SARM1 Hydrolase Buffer to all wells, except the “Blank” wells.

4. Add 20 µl of 1X SARM1 Hydrolase Buffer to the “Blank” wells.

5. Prepare the Test Inhibitor (2.5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

   5.1 If the Test Inhibitor is water-soluble, prepare serial dilutions 10-fold more concentrated than the desired final concentrations in 1x SARM1 Hydrolase Buffer.

   For the positive and negative controls, use 1x SARM1 Hydrolase Buffer (Diluent Solution).

   OR

   5.2 If the Test inhibitor is soluble in DMSO, prepare the inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired concentration, then dilute the inhibitor 10-fold in 1x SARM1 hydrolase buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%. Use 10% DMSO in 1x SARM1 Hydrolase Buffer (vol/vol) for the serial dilution to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in 1x SARM1 Hydrolase Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

6. Add 2.5 µl of diluted test compound to each well labeled as “Test Inhibitor”.

7. Add 2.5 µl of Diluent Solution to the wells labeled “Positive Control" and “Blank”.

8. Prepare the Inhibitor Control by diluting DSRM-3716 (100 mM) to 1000 x the IC_{50} in 100% DMSO. Then dilute 10-fold in 1x SARM1 Hydrolase Buffer (the DMSO amount is now 10%) and corresponds to 100 x the IC_{50} value (2.5 µl/well). Using Diluent Solution prepare solutions at 1 x and 10 x the IC_{50} value (2.5 µl/well).

9. Add 2.5 µl of DSRM-3716 to the “Negative Control” wells.

10. Thaw **SARM1** enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube.

11. Dilute enzyme to 30 ng/µl with 1x SARM1 Hydrolase Buffer (5 µl/well).

12. Add 5 µl of diluted SARM1 enzyme to the wells designated “Positive Control” and "Test Inhibitor”.

13. Cover the plate and incubate 30 minutes at Room Temperature (RT) with gentle agitation.

14. Dilute **e-NAD** 10-fold with 1x SARM1 Hydrolase Buffer.

    Note: Dilute only the amount required for the assay. Store remaining e-NAD at -20°C in single use aliquots (minimum volume of 5 µl). Discard any unused diluted e-NAD after use.

15. After the 30 minute pre-incubation, initiate the reaction by adding 2.5 µl of diluted e-NAD (1.2 mM) to each well.

Component	**Blank**	**Positive Control**	**Negative Control**	**Test Inhibitor**
1x SARM1 Hydrolase Buffer	20 µl	15 µl	15 µl	15 µl
Test Inhibitor	-	-	-	2.5 µl
Diluted DSRM-3716	-	-	2.5 µl	-
Diluent Solution	2.5 µl	2.5 µl	-	-
Diluted SARM1 (30 ng/µl)	-	5 µl	5 µl	5 µl
Incubate 30 minutes at Room Temperature				
Diluted ε-NAD (1.2 mM)	2.5 µl	2.5 µl	2.5 µl	2.5 µl
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**

16. Cover the plate with aluminum foil and incubate for 4 hours with gentle agitation at RT.

17. After incubation, measure the plate using a fluorescence plate reader capable of excitation at 300 nm and detection of emitted light at 410 nm.

18. The “Blank” value should be subtracted from all other values.

Example Results

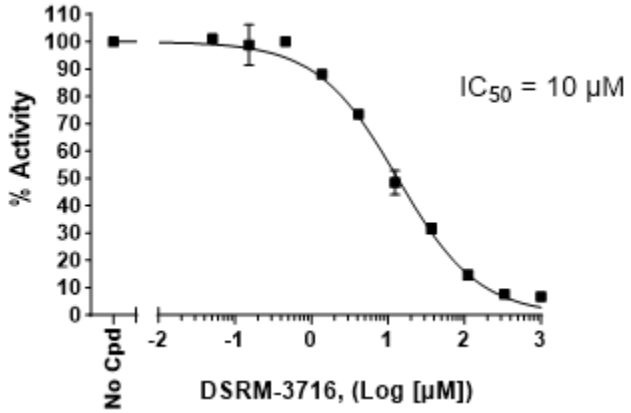

Figure 2: SARM1 inhibition by DSRM-3716.
SARM1 activity was measured in the presence of increasing concentrations of DSRM-3716. Results are expressed as percentage of activity relative to the positive control (measured in the absence of inhibitor and set at 100%). Fluorescence was measured using a Bio-Tek microplate reader.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

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

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