Description

The SARM1 Activation (Base Exchange Activity) Assay Kit is designed to measure SARM1 (Sterile Alpha and TIR Motif Containing 1) enzymatic activity for screening and profiling applications. The SARM1 assay kit comes in a convenient 96-well format, with enough recombinant human SARM1 enzyme (amino acids 28-724 (end)), substrate, fluorescent probe PC6, and SARM1 assay buffer for 100 enzyme reactions.

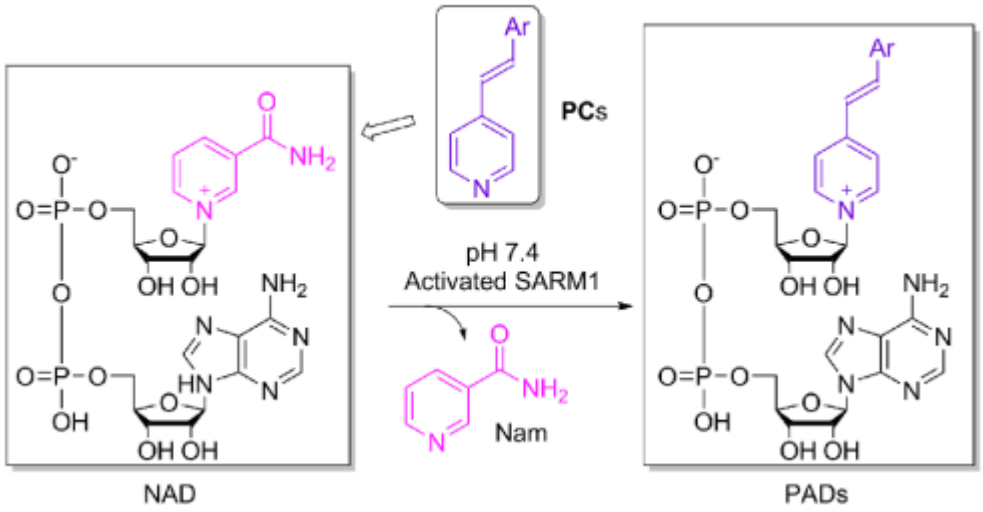

Figure 1: Assay principle

This assay utilizes a synthesized fluorescent PC6 probe (conjugate of styryl derivative with pyridine) to serve as substrate of SARM1, with NAD as the acceptor of base-exchange and nicotinamide mononucleotide (NMN) as an activator. The conversion of PC6 to the exchange product, PAD6 induces a shift in fluorescence from the PC6 emission maximum of 430 nm to the PAD6 emission maximum of 520 nm. Thus, fluorescence intensity at 520nm increases proportionally to the activity of SARM1. (Figure from Li W., *et al.*, 2021 *eLife* 10:e67381)

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.

BPS Bioscience
• bpsbioscience.com • 858-202-1401 • support@bpsbioscience.com

Supplied Materials

Catalog #	Name	Amount	Storage
100069-KC10	SARM1, FLAG-Tag*	10 µg	-80°C
83678-KC3	5x SARM1 Buffer 2	3 ml	-20°C
82735-KC200	0.5 M DTT	200 µl	-80°C
83866-KC250	6 mM β-NMN	250 µl	-20°C
83679-KC250	SARM1 Substrate (1 mM NAD)	250 µl	-20°C
83680-KC13	Lyophilized PC6 Probe	13 µg	-80°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 able to excite at 390 nm and detection of emitted light at 520 nm
- Rotating or rocker platform
- 100% Ethanol for reconstitution of the probe

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.

Contraindications

- The final concentration of DMSO in the assay should not exceed 1%.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound with the assay results.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include “Blank”, “Positive Control” and “Test Activator” 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 β-NMN as internal control. If not running a dose response curve for the control activator, we recommend running the control activator at 0.1 x, 1 x and 10 x the AC_{50} value shown in the validation data below.

1. Thaw **5x SARM1 Buffer 2** and **0.5 mM DTT** on ice.

2. Dilute **0.5 M DTT** 100-fold with **5x SARM1 Buffer 2,** this makes **Complete 5x SARM1 Buffer 2.**

3. Prepare **1x Assay Buffer** by diluting 5-fold **Complete 5x SARM1 Buffer 2** with distilled water.

   Note: Prepare only enough Complete 5x SARM1 Buffer 2 as required for the assay. Store the remaining 5x SARM1 Buffer 2 at -20°C in single-use aliquots.

4. Add 25 µl of **1x Assay Buffer** to all wells, except the "Blank" wells.

5. Add 35 µl of **1x Assay Buffer** to the "Blank" wells.

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

7. Dilute **SARM1** enzyme to 10 ng/µl with 1x Assay Buffer (10 µl/well).

8. Add 10 µl of **diluted SARM1** enzyme to the wells designated "Positive Control" and "Test Activator".

9. Prepare the **Test Activator** (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 50 µl.

   9.1 If the Test Activator is water-soluble, prepare serial dilutions 10-fold more concentrated than the desired final concentrations in 1x Assay Buffer.

   For the positive controls, use 1x Assay Buffer (Diluent Solution).

   OR

   9.2 If the Test Activator is soluble in DMSO, prepare the Activator in 100% DMSO at a concentration 100-fold higher than the highest desired concentration, then dilute the inhibitor 10-fold in 1x Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Use 10% DMSO in 1x Assay Buffer (vol/vol) for the serial dilution to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in 1x Assay 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%.

10. Add 5 µl of diluted **Test Activator** to each well labeled as "Test Activator".

11. Add 5 µl of **Diluent Solution** to the wells labeled "Blank" and "Positive Control".

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

13. Reconstitute **Lyophilized PC6 Probe** by adding 60 µl of 100% Ethanol. This creates a 1 mM solution of PC6 Probe.

14. Prepare a **Master Mix** (10 µl per well): N wells x (2.5 µl of SARM1 Substrate (1 mM NAD) + 0.5 µl of 1 mM PC6 Probe + 7 µl 1X Assay Buffer).

15. After the 30-minute pre-incubation, initiate the reaction by adding 10 µl of **Master Mix** to each well.

Component	**Blank**	**Positive Control**	**Test Activator**
1x Assay buffer	35 µl	25 µl	25 µl
Diluted SARM1 (10 ng/µl)	-	10 µl	10 µl
Test Activator	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Incubate 30 minutes at Room Temperature			
Master Mix	10 µl	10 µl	10 µl
Total	**50 µl**	**50 µl**	**50 µl**

16. Protect your samples from direct exposure to light, mix briefly and incubate at RT for 40 minutes or perform kinetic analysis.

    Note: For kinetic analysis use kinetic mode with a recommended kinetic interval of 3-5 minutes.

17. Read the plate in a fluorescent plate reader capable of excitation at 390 nm and detection of emitted light at 520 nm.

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

Example Results

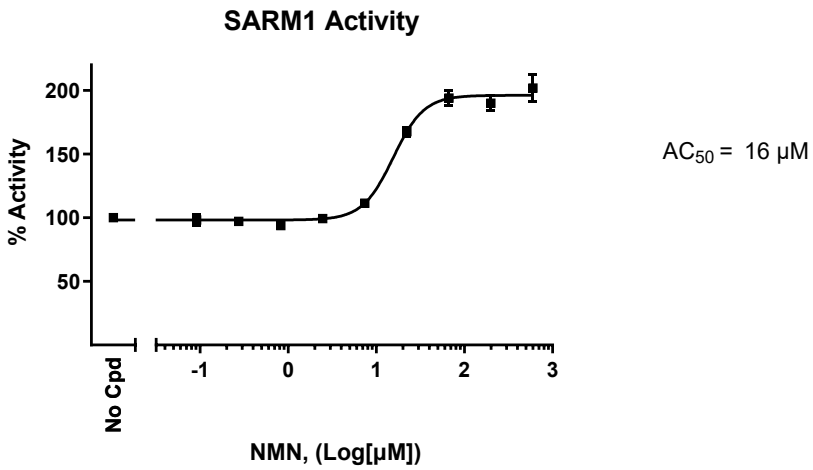

Figure 2: SARM1 activation by β-NMN.
SARM1 activity was measured in the presence of increasing concentrations of β-NMN. Results are expressed as percentage of activity relative to the basal activity (measured in the absence of activator and set at 100%). Fluorescence was measured using a Bio-Tek microplate reader.

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References

Li W., *et al.*, 2021 *eLife* 10:e67381

Hou Y.N., *et al.*, 2022 *Nat Commun.* 13(1):7898.

Related Products

Products	*Catalog #*	*Size*
SARM1 Inhibition (Base Exchange Activity) Assay Kit	83677	96 reactions
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

Version 251204