Description

The Sortase A Assay Kit is a fluorogenic assay kit designed to measure Sortase A proteolytic activity for screening and profiling applications. The Sortase A Assay Kit comes in a convenient 96-well format, with enough purified recombinant Sortase A, Triglycine, Abz/DNP substrate, and assay buffer for 96 enzyme reactions.

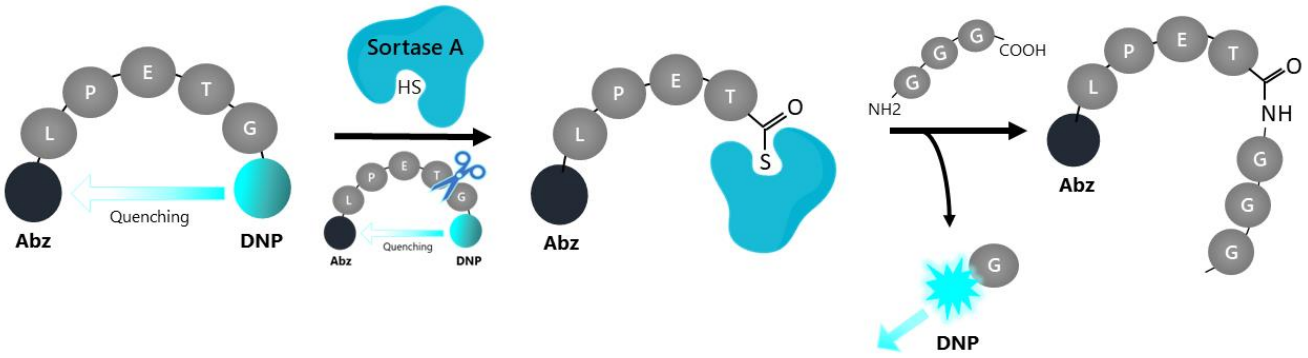

Figure 1: Illustration of the assay principle.

The fluorescence emitted by donor DNP is quenched due to the proximity of the Abz acceptor in the intact peptide. The protease cleaves the peptide between T (threonine) and G (glycine) and forms an intermediate complex between the cysteine residue within the active site and the C-terminus of the peptide substrate. The cleaved substrate is then covalently bounded to the triglycine nucleophile. The glycine-DNP fragment is released, freeing the emission of fluorescence by DNP.

Background

Staphylococcal Sortase A is a bacterial transpeptidase that covalently attaches proteins to the bacterial cell wall, maintaining bacterial virulence and infectivity. Sortase A cleaves a specific peptide sequence (LPXTG recognition motif) within a target protein between threonine and glycine. The cysteine residue of the active site forms a transient thioacyl intermediate complex with the substrate protein. This intermediate complex and substrate is then immediately attacked by oligo-glycine nucleophiles present on peptide-glycans of the bacterial wall to form an amide bond.

Since Sortase A is a critical enzyme needed to maintain the infectivity of the bacterium, it represents a promising therapeutic target. Especially for the treatment of infections caused by antibiotic-resistant bacteria.

Applications

- Screen small molecules inhibitors of Sortase A in high-throughput applications
- Titer inhibitors of Sortase A

Supplied Materials

Catalog #	Name	Amount	Storage	
71086	Sortase A, His-Tag*	12.5 µg	-80°C	***Avoid multiple freeze/ thaw cycles***
79938	2x Sortase Assay Buffer	2.5 ml	-20°C	
79939	Triglycine	250 µl	-20°C	
79940	Abz/DNP substrate	250 µl	-20°C	
79685	Low binding, black NUNC 96-well plate	1	Room Temp	

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

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Materials Required but Not Supplied

- Microplate reader capable of reading fluorescence
- Adjustable micropipettor and sterile tips
- 30°C incubator

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed. ***Avoid multiple freeze/ thaw cycles!***

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 performed in duplicate
- The assay should include a "Blank" and a "Positive control"
- The final concentration of DMSO should not exceed 1%.

1. Thaw 2x Sortase assay buffer, Triglycine, and Abz/DNP substrate. Briefly spin the tubes to recover the full content.

2. Prepare 5 ml of 1x Sortase assay buffer by mixing 2.5 ml of stock 2x Sortase Assay buffer with 2.5 ml of distilled water. Note that 5 ml of 1x Sortase Assay buffer is sufficient for 100 reactions.

3. Prepare a Master Mix (25 µl/well): N wells x (20 µl of 1x Sortase Assay buffer + 2.5 µl of Triglycine + 2.5 µl of Abz/DNP substrate).

4. Prepare the Test Inhibitor (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.

   Without DMSO

   a. If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x Sortase Assay Buffer, 10-fold more concentrated than the desired final concentrations. For the positive control, use 1x Sortase Assay Buffer (Diluent Solution).

   OR

   With DMSO

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

   b. Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in 1x Sortase Assay Buffer to keep the concentration of DMSO constant.

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

5. Add 25 µl of Master Mix to each well, including "Blank", "Positive Control" and "Test Inhibitor".

6. Add 5 µl of Inhibitor dilution to each well labeled as "Test Inhibitor."

7. Add 5 µl of Diluent Solution to the "Positive Control" and "Blank".

8. Add 20 µl of 1x Sortase Assay Buffer to the "Blank"

9. Thaw **Sortase A** on ice. Briefly spin the tube containing the enzyme to recover the full contents. If not using the entire plate, calculate the amount of Sortase A required. Dilute the enzyme to 6.25 ng/µl with 1x Sortase A assay buffer (20 µl/well). Aliquot unused protein into 2-4 aliquots as may be necessary (single use aliquots) and store them at -80°C.

Note: Sortase A is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. ***Do not re-use*** *the diluted Sortase A.*

10. Initiate the reaction by adding 20 µl of diluted Sortase A to the wells designated "Positive Control" and "Test Inhibitor".

	Blank	**Positive Control**	**Test Inhibitor**
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	–	–	5 µl
Diluent Solution (no Inhibitor)	5 µl	5 µl	–
1x Sortase Assay Buffer	20 µl	–	–
Sortase A (6.25 ng/µl)	–	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

11. Incubate at 30°C for 30 minutes.

12. Read fluorescence at λex=320 nm and λem=420 nm. "Blank" value is subtracted from all measurements.

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Example Results:

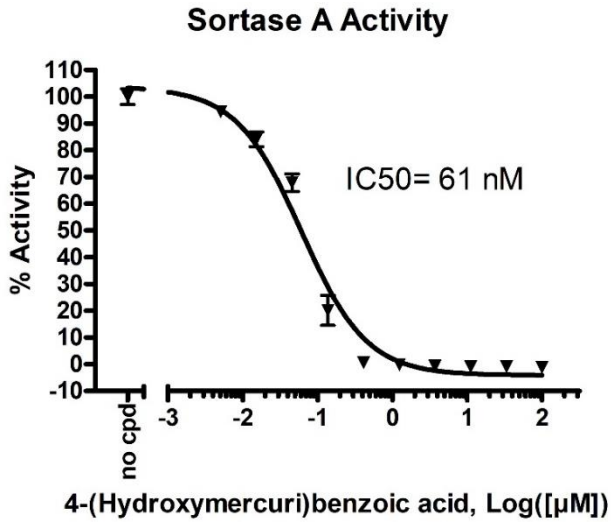

Figure 1: Inhibition of Sortase A activity.
Sortase A activity was measured in the presence of increasing concentrations of 4-(Hydroxymercuri)benzoic acid using the Sortase Assay Kit.

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

References

1. Mazmanian S.K., *et al.* 1999. "Staphylococcus aureus sortase, an enzyme that anchors surface proteins to the cell wall." *Science* **285(5428)**: 760-763.
2. Spirig T. *et al.* 2011. "Sortase enzymes in Gram-positive bacteria." *Molecular Microbiology* **82(5)**: 1044-1059.

Related Products

Products	*Catalog #*	*Size*
Sortase A, S. aureus	71086	50 µg
Sortase A, Pentamutant	71046	50 µg
Sortase A, Hexamutant	71047	50 µg
Sortase A, Octamutant	72518	50 µg
Sortase A, Heptamutant,	71048	50 µg
Sortase Sampling Kit	79709	50 µg
SiMPLe Protein Labeling Kit	79392	3 Units

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