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Data Sheet

Sortase A Assay Kit

Catalog #79937
96 Reactions

DESCRIPTION: The *Sortase A Assay Kit* is designed to measure Sortase A 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.

BACKGROUND:
Staphylococcal Sortase A is a bacterial transpeptidase that covalently attaches proteins to the bacterial cell wall by cleaving between threonine and glycine at an LPXTG recognition motif. It catalyzes the formation of an amide bond between the carboxyl-group of threonine and the amino-group of the cell-wall peptidoglycan, which is an essential procedure for pathogens to sustain infection. Thus, Sortase A has potential as an anti-infective therapy target, especially for antibiotic-resistant bacteria.

COMPONENTS:

Catalog #	Reagent	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	Black, low binding NUNC black microtiter plate	1	RT	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Microplate reader capable of reading luminescence
Adjustable micropipettor and sterile tips
30°C incubator

APPLICATIONS: Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months when stored as recommended.

REFERENCE:
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.

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Thaw **2x Sortase assay buffer**, **Triglycine,** and **Abz/Dnp substrate**.

2) Prepare 5 ml of **1x Sortase assay buffer** by mixing 2.5 ml of **2x Sortase assay buffer** with 2.5 ml water. 5 ml of **1x Sortase assay buffer** is sufficient for 100 reactions.

3) Prepare the master mixture (25 µl per well): N wells x (20 µl **1x Sortase assay buffer** + 2.5 µl **Triglycine** + 2.5 µl **Abz/Dnp substrate**). Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
1x Sortase assay buffer	20 µl	20 µl	20 µl
Triglycine	2.5 µl	2.5 µl	2.5 µl
Abz/Dnp substrate	2.5 µl	2.5 µl	2.5 µl
Test Inhibitor	–	5 µl	–
10% DMSO in Water (inhibitor buffer)	5 µl	–	5 µl
Sortase A, His-Tag (6.25 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

4) Add 5 µl of Inhibitor solution of each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 5 µl of the same solution without inhibitor (Inhibitor buffer; usually 10% DMSO in water). *Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor dissolved in 100% DMSO at 10 µM, dilute 1 mM inhibitor with water to make a 100 µM inhibitor in 10% DMSO(aq). Then, add 5 µl of the 100 µM solution into the 50 µl assay to make a 1% DMSO concentration in the final reaction mixture.*

5) To the wells designated as "Blank," add 20 µl of **1x Sortase assay buffer**.

6) Thaw **Sortase A His-Tag** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **Sortase A, His-Tag** required for the assay and dilute enzyme to 6.25 ng/µl with **1x Sortase A assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: Sortase A, His-Tag is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted Sortase A.*

7) Initiate reaction by adding 20 µl of diluted **Sortase A His-Tag** to the wells designated “Positive Control” and "Test Inhibitor." Incubate at 30˚C for 30 minutes.

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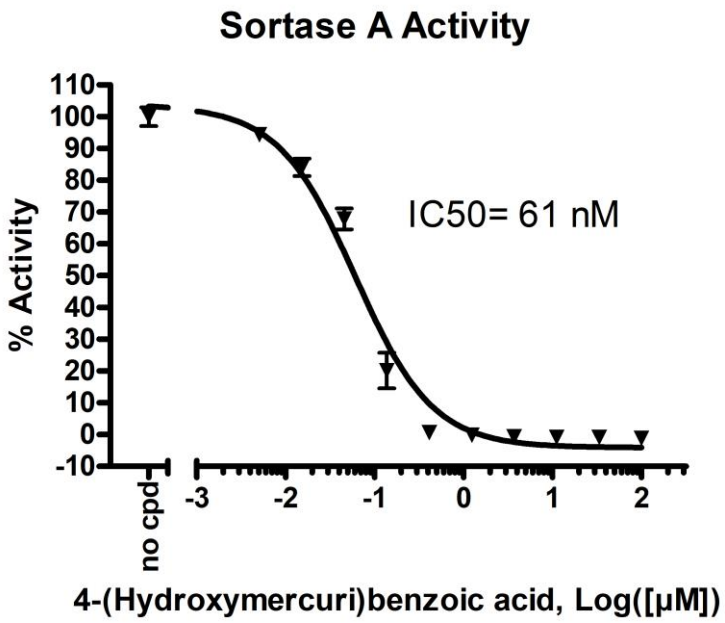
8) Read fluorescence at λex=320 nm and λem=420 nm. "Blank" value is subtracted from all measurements.

Example of Assay Results:



Inhibition of Sortase A, His-Tag by 4-(Hydroxymercuri)benzoic acid, measured using the Sortase A assay kit (BPS Bioscience, #79937). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	**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 A, Hexamutant,	71047	50 µg
Sortase Sampling Kit	79709	50 µg
SiMPLe Protein Labeling Kit (Sortase Mediated Protein Ligation)	79392	3 units

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