

Recombinant Sortase A5 protein

Catalog No: 13100, 13101

Expressed In: *E. coli*

Quantity: 50, 250 µg

Concentration: 1 µg/µl

Source: *S.aureus*

Buffer Contents: Sortase A5 protein expressed in *E. coli* and provided at 1 mg/ml in 50 mM HEPES pH 7.5, 150 mM NaCl and 20% glycerol.

Sortase A5 protein labeling set – 50 µg (Cat. No. 13100), includes:

- Sortase A5 – 50 µg
- Reaction Buffer - 1 ml
- Stop Solution - 150 µl

Sortase A5 protein labeling set – 250 µg (Cat. No. 13101), includes:

- Sortase A5 – 250 µg
- Reaction Buffer – 5 x 1 ml
- Stop Solution – 5 x 150 µl

Background: Sortase belongs to a class of transpeptidases that utilize an active site cysteine thiol to modify proteins by recognizing and cleaving a carboxy-terminal sorting signal, LPXTG (where X is any amino acid), between the threonine and glycine residues. **Sortase A5** is an engineered pentamutant variant of the wild-type sortase from *Staphylococcus aureus* that is significantly more active than the wild-type sortase. Sortase A5 site-specifically labels antibodies or proteins when the LPXTG recognition sequence is displayed. Easily attach a wide variety of labels such as peptides, DNA, carbohydrates or fluorophores containing a poly-Glycine sequence (Gly)_n (where n = 3 or more Glycine residues). **Sortase A5 Pentamutant is covered by US patent number 9,267,127.**

Protein Details: Recombinant Sortase A5 protein (*S. aureus*, Uniprot A0A077UNB8-1), containing amino acid substitutions P94R, D160N, D165A, K190E and K196T, was expressed in *E. coli* and includes a C-terminal 6X His-tag. The M.W. of the protein is 17.8 kDa.

Protein Sequence:

MQAKPQIPKDKSKVAGYIEIPDADIKEPVYPGPATREQLNRGVSFAEENESLDDQNISIAGHTFIDRPNYQFTNLKAAK
KGSVMVYFKVGNETRKYKMTSIRNVKPTAVGVLDEQKKGDKQLTLITCDDYNEETGVWETRKIFVATEVKLEHHHHHH

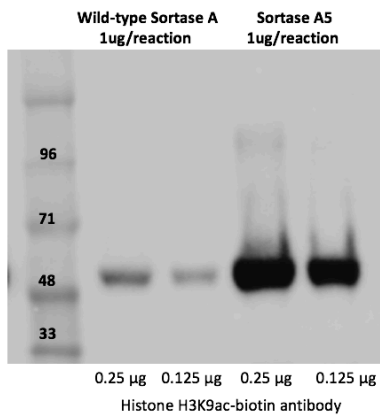
Application Notes: Sortase A5 recognizes an antibody or protein genetically engineered to contain the LPXTG motif (where X is any amino acid). Sortase A5 cleaves this sequence between the threonine and glycine residues and the terminal glycine is then replaced with any poly-Glycine (G)_n label. Sortase A5 is used in Active Motif's Sortag-IT™ Labeling Kits to attach HRP, biotin, fluorophores and other labels directly to Active Motif's AbFlex™ recombinant antibodies (rAb). The activity of both wild type and Sortase A5 pentamutant proteins are Ca²⁺ dependent, therefore, Active Motif's Sortag-IT labeling buffers and reagents are formulated to contain Ca²⁺ for optimal protein labeling. In addition, our AbFlex recombinant antibodies are provided in HEPES buffer, which does not bind Ca²⁺ and will not interfere with the Sortase A5 enzymatic activity.

Storage and Guarantee: Stored at -80°C to prevent degradation and avoid repeated freeze/thaw cycles. This product is guaranteed for 6 months from date of arrival.



Sortase A5 protein gel.

Sortase A5 run on an SDS-PAGE gel and stained with Coomassie Blue.



Sortase A5 has increased labeling efficiency compared to the Wild-Type sortase.

The H3K9Ac AbFlex antibody (67 µg) was labeled with 5 µg Biotin (1.28 mM) using 1 µg Sortase A5 or Wild-Type sortase at 30 °C for 2 hr. with shaking.

Top Panel: Following purification, 0.25 and 0.125 µg of labeled antibodies were run on an SDS-PAGE PAGE gel and labeling was detected using streptavidin-HRP. Lanes 1 & 2: 0.25 µg and 0.125 µg of H3K9Ac AbFlex Ab labeled with wild-type Sortase, respectively. Lanes 3 & 4: 0.25 µg and 0.125 µg of H3K9Ac AbFlex Ab labeled with Sortase A5, respectively.

Bottom panel: The intensity of the labeling was quantified using BioRad's Gel Doc imaging system, represented here as arbitrary units.

