Recombinant Sortase (2A.9) protein

Catalog No: 13112
Expressed In: *E. coli*

Quantity: 50 µg
Concentration: 1 µg/µl
Source: *S. aureus*

Buffer Contents: Sortase 2A.9 protein expressed in *E. coli* and provided at 1 mg/ml in 25 mM Tris, pH 7.5, 150 mM NaCl, 5 mM DTT, and 5% Glycerol.

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 2A.9** is an engineered variant of the wild-type sortase from *Staphylococcus aureus* that is significantly more active than the wild-type sortase for site-specific labeling of antibodies or proteins containing an LAXTG recognition sequence (where X is any amino acid). 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 2A.9 is covered by US Patent No. 10,202,593.**

Protein Details: Recombinant Sortase 2A.9 protein containing the following amino acid substitutions relative to Sortase A5 pentamutant (*S. aureaus*, Uniprot A0A077UNB8-1): S102C, A104H, E105D, K138P, K152I, N160K, K162H, T164N , K173E, I182V, T196S. The protein was expressed in *E. coli* and includes a C-terminal 6xHis-Tag.

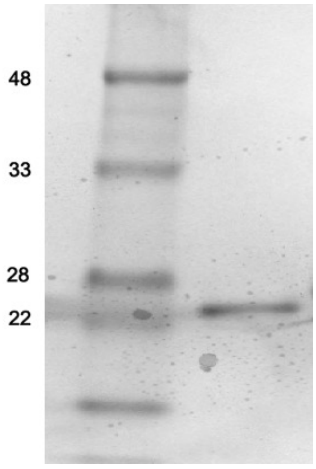
Protein Sequence:
MQAKPQIPKDKSKVAGYIEIPDADIKEPVYPGPATREQLNRGVCFHDENESLDDQNISIAGHTFIDRPNYQFTNLKAAK
PGSMVYFKVGNETRIYKMTSIRKVHPNAVEVLDEQEGKDKQLTLVTCDDYNEETGVWESRKIFVATEVKGSHHHHHH

The protein has a calculated molecular weight of 17.8 kDa and an observed molecular weight of approximately 20 kDa.

Application Notes: Sortase 2A.9 recognizes an antibody or protein genetically engineered to contain the LAXTG motif, where X is any amino acid. Sortase 2A.9 cleaves this sequence between the threonine and glycine residues and the terminal glycine is then replaced with any poly-Glycine (G)n label to attach HRP, biotin, fluorophores and other labels. Sortase A2.9 may be used in a variety of labeling applications and conditions. Some suggested buffers for use are as follows:

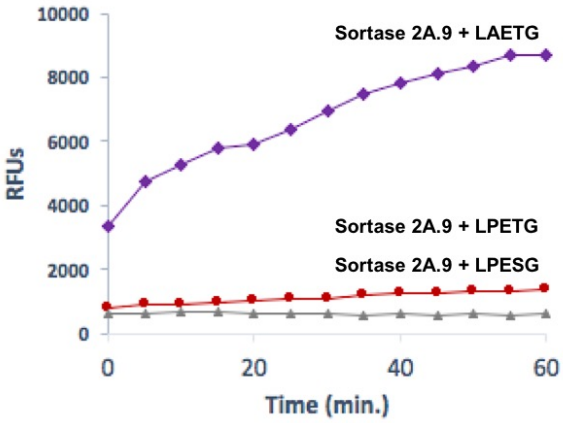
Reaction Buffer: 300 mM Tris-HCl, 150 mM NaCl, 5 mM CaCl2, pH 7.5
Stop Solution: 500 mM EGTA, pH 8.0.

Storage and Guarantee: Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage.



Recombinant Sortase 2A.9 SDS-PAGE gel
1.5 µg of Sortase 2A.9 protein was run on a 4% to 12% gradient gel, followed by staining with Coomassie blue.

Calculated MW: 17.8 kDa
Observed MW: 20 kDa



Recombinant Sortase (2A.9) protein specificity for LAETG sequence
Sortase (2A.9) at 12 ng/µl was incubated with 0.4 mM LAETG, LPETG or LPESG substrates in reaction buffer, followed by fluorescence detection upon cleavage. Results demonstrate the high specificity of Sortase (2A.9) for LAETG. The 2A.9 shows minimal activity to the WT substrate LPETG and no activity towards the mutated substrate LPESG.