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Sortase A

Sortase A

(Staphylococcus aureus)

- Transpeptidase / Protein-Ligase -

Cat. No.	Size
E4400-01	20 µg

Package Contents:

- 1 µg/µl Sortase A
- Sortase A 10x Reaction Buffer

1 x Reaction Buffer:
50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 10 mM $CaCl_2$, 4 mM β-mercaptoethanol.

Modifications:

- Contains C-terminal His6-Tag.
- Truncated (lacks N-terminal membrane-anchoring motif).

Source:

- Recombinant from *Staphylococcus aureus*.
- Cloned and expressed in *Escherichia coli*.

Storage Conditions: Store at -20°C
Protein is stable at least for 9 months.

Reaction Temperature: 20 - 50° C

Sortase A exhibits transpeptidase ("protein ligase") as well as protease activities, respectively.

Description:

- Sortase A is a bifunctional protein displaying both protein ligase / transpeptidase and cysteine protease activities (4).
- Facilitates protein ligation under mild, physiological reaction conditions. Allows site-specific posttranslational generation of fusion proteins and of non-peptidic bioconjugates, which are not accessible by standard molecular cloning techniques.
- Sortase A tolerates incorporation of oligoglycine residues containing C-terminal synthetic substituents. Thus, Sortase A permits ligation of peptides to native and non-native peptidic molecules, to proteinogenic and non-proteinogenic ("unnatural") amino acids, as well as to non-peptidic functional groups, such as nucleic acids, fluorophores, dyes, antibiotics and sugar derivatives (see 2, 3).
- Applicable for immobilization of proteins to solid surfaces (5), for cell-surface labelling of living organisms (1), for protein circularization (6), and for posttranslational incorporation of novel functionality in proteins which cannot be genetically encoded and thus are not accessible via molecular cloning techniques (reviewed in 2,3).
- Sortase recognizes solvent-exposed LPXTG recognition motifs and cleaves between the threonine and glycine residues, respectively.
- Proteins with an accessible (solvent-exposed) LPXTG recognition motif are substrates. Target peptides carry one (or more) solvent-exposed N-terminal glycine residues, serving as nucleophile. Efficient targets are oligoglycine probes, which may carry C-terminal functional groups.
- The recognition sequence LPXTG may be located either at C-termini or within flexible loops of substrate proteins, as long as the recognition site is sterically accessible to the enzyme, i.e. solvent-exposed . For efficient cleavage, the glycine of the LPXTG recognition motif must carry a peptide bond to at least one additional amino acid. The minimal C-terminal recognition sequence with full functionality is LPETGG (1).
- Sortase cleavage products serve as substrates for ligation. Both, protein ligase and protease activities cannot be separated. Consequently, sortase A mediated reactions reach an assay-specific equilibrium between ligation and cleavage activities.
- Ligation efficiency is dependent on the structure and the concentration of both substrate and target proteins. Fusion protein yield is highly assay-dependent and may vary from a few percent up to 90%.
- N-terminal protein labeling is possible by moving the LPXTG recognition site from the substrate protein to the C-terminus of the short peptide probe (3).

Quality Control:

Sortase A is greater than 95% single-band pure without non-specific protease contamination.

References:

1. *Popp M. et al. (2009) Curr. Protoc. Protein Sci. 56: 15.3.1-15.3.9.*
2. *Proft T. (2010) Biotechnol Lett. 32(1):1-10.*
3. *Popp M. and Ploegh H. (2011) Angew. Chem. Int. Ed. 50, 5024 – 5032*
4. *Mao H. et al. (2004), J Am Chem Soc 126:2670-2671*
5. *Parthasarathy R. et al. (2007) Bioconjug Chem 18: 469-476*
6. *Antos J.M. et al. (2009) J Biol Chem 284: 16028-16036*
7. *Ton-That H. et al. (1999) PNAS 96, 22: 12424-12429*
8. *Williamson D. J. et al., (2012), Angew. Chem. Int. Ed., 51, 9377 – 9380*

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PROTEIN LIGATION PROTOCOL

Example Protocol:

Protein- Protein Ligation:

Sortase A mediated protein ligation requires a substrate protein with a C terminal LPET motif and a target protein determined for ligation with two or three N-teminal glycine residues. Both termini must be solvent-exposed and must be sterically accessible to sortase A.

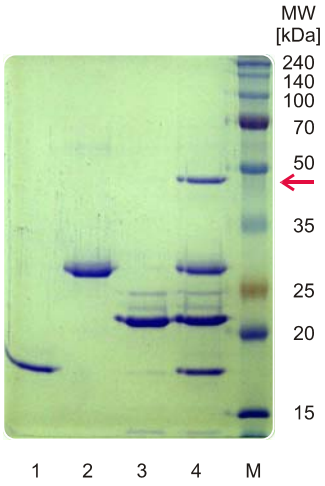

Fig. 1: Example reaction of sortase A activity. SDS PAGE gel.
Lane 1: rnpA protein with C-terminal partial sortase A recognition sequence LPET (= substrate protein),
Lane 2: GFP with N-terminal GGG generated by TEV (Cat.No. E4310) cleavage (= target protein),
Lane 3: Sortase A protein, 1 µg,
Lane 4: all components from line 1,2,3 incubated for 60 min at 30˚C in 1x reaction buffer.
The position of the fusion protein in lane 4 is marked with a red arrow.
Marker: Perfect Color Protein Ladder (Cat.No. E3215).

Example Reaction (20µl):

2 µl 10x Sortase A buffer
X µl substrate protein A (with Sortase recognition sequence)
Y µl target protein B (with N-terminal glycine / nucleophile)
0.1 - 1 µg Sortase A
H_2O up to 20 µl

Incubate 60 min at 30˚C.
Detection of sortase ligation by SDS PAGE gel electrophoresis.

Note 1: The efficiency of ligation depends on the concentrations of substrate and target proteins, on their concentration ratios as well as on the amount of sortase A. Highest ligation efficiencies are obtained during prolonged incubation times (up to 8 hours). Optimal reaction conditions for sortase A are provided in a pH range between 7.5 - 9.0 and in a temperature range between 20˚C and 50˚C, respectively. Alternate reaction buffers must not contain any primary amine derivatives such as hydroxylamine.

Note 2: There exists no generally applicable set of reaction parameters fitting to each and every posttranslational ligation assay. The optimal ligation conditions vary with the nature, the conformation and the structure of substrate and target proteins, respectively. Thus each newly developed assay requires experimental optimization of reaction parameters.

Note 3: The process is reversible, since ligation continuously regenerates the recognition motif (8). The process may become irreversible, as soon as the recognition motif becomes inaccessible for sortase A due to structural changes within the newly generated fusion protein.

Sortase A
PRIMER DESIGN

PCR Primer Design for Inclusion of a Sortase Cleavage Site at C-Termini.

Sortase removes C-termini of proteins. Thus, cleavage sites must be incorporated at the 3'-end of the coding sequence. For introduction of cleavage sites via PCR amplification, the (reverse of) the extension given below has to be added to the 3'-end of the target gene sequence and must be 3'-extended with a gene-of-interest-specific priming sequence.

The gene sequence given below is optimized for E. coli codon usage. Other hosts may require further adjustment to their specific codon usage requirements.

Gly4Lys linker sequence and HA epitope tag are recommended, but optional features. The optional Gly4Lys linker ensures the accessibility of the cleavage site to Sortase A. It may prove necessary to vary the linker size for certain proteins to ensure full exposure of the LPETGG recognition motif to the solvent.

The optional HA epitope tag is cleaved off after sortase treatment for monitoring cleavage efficiency via immunoblotting. Alternate epitope tags, such as the BirA Acceptor Peptide sequence (MAGGLNDIFEAQKIEWHEDTGGA) may be used to replace the HA epitope tag.

An optional C-terminal His6-Tag (Codons: CAT or CAC, six repeats; not included in the sequence below) inserted between epitope tag and stop codon aids in protein purification and removal of cleaved-off C-termini and non-processed target protein along with Sortase A on Ni-NTA columns.

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    G   G   G   G   S     L   P   E   T   G   G     Y   P   Y   D   V   P   D   Y   A     *
   Gly Gly Gly Gly Ser   Leu Pro Glu Thr Gly Gly   Tyr Pro Tyr Asp Val Pro Asp Tyr Ala   Stop
5'- GGC GGT GGC GGT AGC   CTG CCG GAA ACC GGC GGT   TAT CCG TAC GAT GTG CCG GAT TAT GCG   TAA -3'
3'- CCG CCA CCG CCA TCG   GAC GGC CTT TGG CCG CCA   ATA GGC ATG CTA CAC GGC CTA ATA CGC   ATT -5'
    [      Linker     ]   [   Sortase Cleavage   ]   [          HA Epitope Tag          ]
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Schematic overview of Sortase A substrate design (non-length-proportional sketch):

Sequence for a suitable 5'-primer extension for the gene specific reverse primer (*without* His6-tag, 63 bp, *E. coli* codon usage; a gene specific sequence stretch, 20 bp or longer, remains to be added to the 3'-end):

5'-TTA CGC ATA ATC CGG CAC ATC GTA CGG ATA ACC GCC GGT TTC CGG CAG GCT ACC GCC ACC GCC -3'

His6-tag supplemented sequence for a 5'-primer extension to a gene specific reverse primer (*with* His6-tag, 81 bp; a gene specific sequence stretch, 20 bp or longer, remains to be added to the 3'-end):

Optimized for *Escherichia coli* codon usage:

5'-TTA ATG GTG ATG GTG ATG GTG CGC ATA ATC CGG CAC ATC GTA CGG ATA
ACC GCC GGT TTC CGG CAG GCT ACC GCC ACC GCC -3'

Optimized for *Homo sapiens* codon usage:

5'-TCA ATG GTG ATG GTG ATG GTG GGC GTA GTC GGG CAC GTC GTA GGG GTA
TCC GCC GGT CTC GGG CAG GCT TCC GCC TCC GCC -3'