3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Omniligase™-1
recombinant, from *Bacillus* sp.

Catalog Number **SAE0068**
Storage Temperature –70 °C

Product Description

Omniligase™-1 is a peptide ligase which has very broad substrate specificity. It is a fourth-generation variant of the engineered enzyme peptiligase, which was originally developed specifically for chemo-enzymatic peptide synthesis (CEPS) applications.[1] CEPS, the enzymatic ligation of chemically synthesized peptide segments, is applicable to the synthesis of medium-sized and long peptides (e.g., 20–100 amino acids). CEPS is useful for applications like peptide-to-protein couplings. Features of CEPS include:

- Fast and simple peptide-peptide, peptide-to-protein coupling reactions within minutes in aqueous solution
- An epimerization-free ligation
- Protection of substrate side-chain residues is not required
- A ligation without recognition tag (traceless)
- Simple, efficient peptide cyclization[4]

Omniligase-1 combines properties from different enzymes, namely the hyperstable, cation-independent subtilisin BPN′ variant Sbt149,[2] and of thiolsubtilisins such as subtiligase.[3] Typically, Omniligase-1 catalyzes the ligation between a peptide ester (acyl donor) and a peptide amine (acyl acceptor, nucleophile) segment (see Figures 1, 2, and Appendix, Figure 4).

Omniligase-1 can be used to perform condensation of linear peptide segments (see Figure 1). It also efficiently catalyzes peptide macrocyclization (see Figure 2), as well as conjugation of peptides to proteins.

Figure 1.
Schematic of Linear Peptide Coupling Reaction Mediated by Omniligase-1.

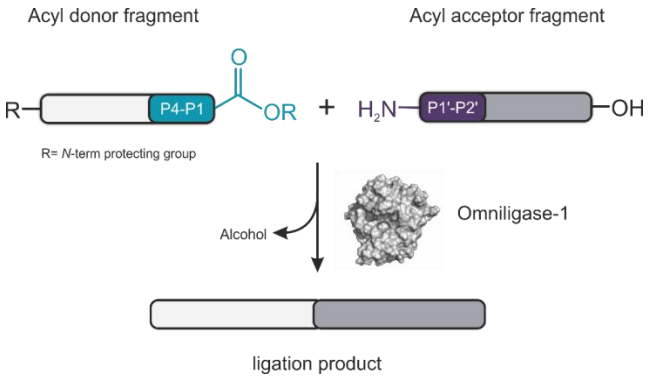
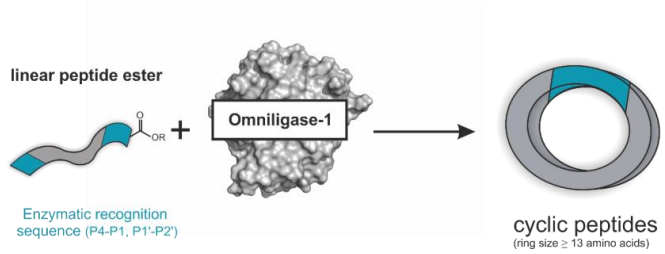


Figure 2.
Schematic of Head-to-tail Peptide Cyclization Mediated by Omniligase-1.



Omniligase-1 has six substrate recognition subsites, as shown in Appendix, Figure 4 (using the nomenclature of Schechter & Berger):

- Four at the acyl donor binding site: S1–S4
- Two at the amine site: S1′ and S2′

The full substrate scope of the enzyme has been determined and is very broad, as shown in Appendix, Figure 5.

Component

Omniligase-1 is provided as a frozen solution of 1 mg/mL in 25 mM Tricine buffer, pH 7.5.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
This product should be stored at ≤ –70 °C. Repeated freeze-thaw cycles should be avoided with this enzyme. It is suggested to aliquot the Omniligase-1 stock solution (e.g., into 5 µL aliquots), and to store the aliquots at ≤ –70 °C or –80 °C in cryovials, or at a minimum, at ≤ –20 °C in 0.2 mL safe-lock vials.

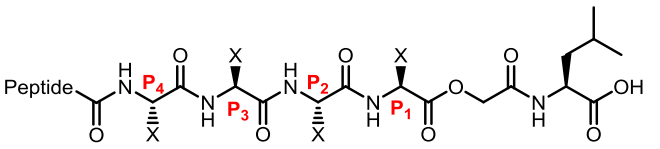
Under reaction conditions, Omniligase-1 is stable at temperatures up to 55 °C, but unfolds at 70 °C. Omniligase-1 is active in various solvent conditions:

- Organic co-solvents (e.g., up to 50% DMF or 50% DMSO)
- Chaotropic agents (up to 4 M guanidinium hydrochloride, 2 M urea)
- Denaturants (e.g. SDS, Triton™ X-100),
- Chelators (EDTA)
- Reducing agents (e.g., DTT [dithiothreitol] and TCEP [tris-(2-carboxyethyl)phosphine hydrochloride])

Procedure
In general, to confirm Omniligase-1 activity, it is recommend to use the Ligation Assay Solution (Catalog Number SAE0070) with the Omniligase-1 (Catalog Number SAE0068).

Substrates – Acyl Donor (Ester Fragment)
Alkyl, aryl, and thioesters can be utilized. However, carboxyamidomethyl (Cam) esters or substituted Cam esters (e.g. –Cam-Leu-OH or –Cam-Leu-NH_2) are recommended. The substitution of the Cam ester can be used to alter the polarity, and thus the solubility of the ester fragment. Cam esters and substituted Cam esters can be generated on resins commonly used for solid-phase peptide synthesis.[3]

Figure 3.
Substituted Cam Ester



Peptide-OCam-Leu-OH ester

Because Omniligase-1 has such broad specificity, the acyl donor (ester) fragment normally requires N-terminal protection to prevent formation of cyclic peptides or polymers. The use of a phenylacetyl protection group is recommended, as it can be removed easily with commercially available acylases.

Substrates – Nucleophile (Amine Fragment)
The nucleophilic (amine) peptide fragment requires no protection at all. All amino acids except prolines in the P1′ and P2′ are accepted.

Reaction:
The reaction is fast, typically taking 5-10 minutes at an Enzyme:Product ratio of ~1:100 (w/w) for a two-fragment coupling. For convenience, most coupling reactions can be performed at ambient temperature. Conversion to the desired product is usually higher than 80% (by HPLC) at a 1.1 to 1.5 molar excess of the amine fragment.

The conversion generally improves with the length of the fragments. The reaction is intrinsically traceless, but can also be used with peptide linkers between other peptides, peptide fragments, or proteins.

Identification of ideal coupling positions:
To identify suitable fragments for a coupling reaction, it is recommended to find a coupling position with a hydrophobic P4 residue and well-recognized amino acids in P1′ and P2′ pockets. Please note that a combination of sub-optimal amino acids can lead to a low coupling yield. In particular, prolines near the coupling site should be avoided.

General rules for obtaining a high yield:
- The concentration of the amine fragment should be as high as possible.
- Always check (and adjust) the pH before starting the reaction.
- Reducing conditions are preferred, i.e., addition of TCEP (or optionally DTT). Oxidative conditions, or the presence of disulfides or maleimides can inhibit the enzyme.

Analysis
For analysis of the coupling reaction by HPLC-MS, it is recommended to use acidic conditions (pH <3) to quench the reaction before the analysis.

Important considerations:

1. Substrate concentration
   The amine fragment concentration is crucial for the reaction yield. A higher concentration often results in a better coupling yield.

2. pH
   A pH between pH 8.0–8.5 is optimal and required for an efficient ligation. At lower pH, the amine fragment is protonated, and thus unreactive. At higher pH, the Cam-ester is chemically hydrolyzed. Because peptides are usually acidic after final TFA (trifluoroacetic acid) deprotection and a preparative HPLC step, it is crucial to check the pH, and carefully adjust the pH if needed, before starting the enzymatic coupling reaction.

3. Temperature
   Omniligase-1 can be used in the temperature range of 4–50 °C. However, ambient temperature is generally preferable.

4. Additives
   Avoid reagents that react with thiols, such as maleimides or iodoacetamides, since they will inhibit the active site cysteine of the Omniligase-1.

5. Head-to-tail cyclizations
   The use of Omniligase-1 with an unprotected ester fragment can be employed to manufacture the corresponding head-to-tail macrocyclic peptide (>13 amino acid ring size) without significant generation of dimers or polymers. Because of the enzyme's broad substrate specificity, most macrocycles can be cyclized at various positions.

6. Compatible reagents
   Table 1 shows compatible concentrations of selected reagents. Higher levels may be acceptable, but those should be tested before use. Please note that some of these reagents may partially or completely denature Omniligase-1.

Table 1.
Compatible Reagents

Reagent	**Compatible Concentration**
TCEP	1–5 mg/mL
DTT	1–5 mg/mL
EDTA	10 mM
SDS	5% (w/v)
Triton X-100	5% (w/v)
Urea	6 M
Guanidinium chloride	2 M

7. Incompatible reagents with the ligation reaction
   - Maleimide
   - Iodoacetamide
   - Epoxides
   - High concentrations of alcohols (risk of transesterification)
   - High concentrations of thiols (risk of thioester synthesis)
   - Ammonia or other primary amines (risk of amide synthesis)

Various coupling protocols

1. Macrocyclization
   Prepare a solution of the linear side-chain unprotected peptide ester (>13 amino acids) at a suitable concentration (0.1–2.0 mg/mL) in 1 M potassium phosphate buffer, pH 8.3, containing 1 mg/mL of TCEP. Check the pH of the resulting reaction mixture, and adjust the pH if necessary. Start the cyclization reaction by addition of the enzyme, and follow the reaction using HPLC-MS.

2. Two-fragment ligation
   Prepare a solution of the linear side-chain unprotected (N-terminal protected, if necessary) peptide ester fragment and the amine fragment to be coupled (1.1-2.0 eq.) at a suitable concentration. (Note that high concentrations give higher reaction yields.) Use a suitable buffer to prepare the solution, e.g., 1 M potassium phosphate buffer, pH 8.3, containing 1 mg/mL of TCEP. Check the pH of the resulting reaction mixture, and adjust the pH if necessary. Start the ligation reaction by addition of the enzyme and follow the reaction using HPLC-MS. When the ligation is completed, the (optional) N-terminal protecting group can be removed to obtain the final product.

3. Troubleshooting tips
   Little or no conversion:
   - Presence of maleimide
   - Check the enzyme activity (with SAE0070)
   - Check if the chosen sequence represents a well-recognized substrate
   - Check that all substrates are in solution
   - Are reductive conditions being used? If not, please note that oxidative conditions can inhibit Omniligase-1.

   Excessive hydrolysis:
   - Check if the amine fragment concentration can be increased. If so, try to perform the reaction at higher concentration.
   - Check the pH.
   - Check if the two amino acids at the P1′ and P2′ positions are sub-optimal. If so, try to choose a different coupling position.

   Special issues related to conjugation to proteins:
   The P1′ and P2′ amino acids may not be accessible. In such cases, one can check if the use of disrupting agents (urea or guanidinium chloride) or a peptide spacer could enhance the coupling efficiency.

References

1. Toplak, A. *et al*., *Adv. Synth. Catal.*, **358(13)**, 2140-2147 (2016).
2. de Beer, R.J. *et al.*, *Org. Biomol. Chem.*, **10(33)**, 6767-6775 (2012).
3. Nuijens, T. *et al.*, *Tetrahedron Letters*, **57(32)**, 3635-3638 (2016).
4. Schmidt, M. *et al*., *Adv. Synth. Catal.*, **359(12)**, 2050-2055 (2017).

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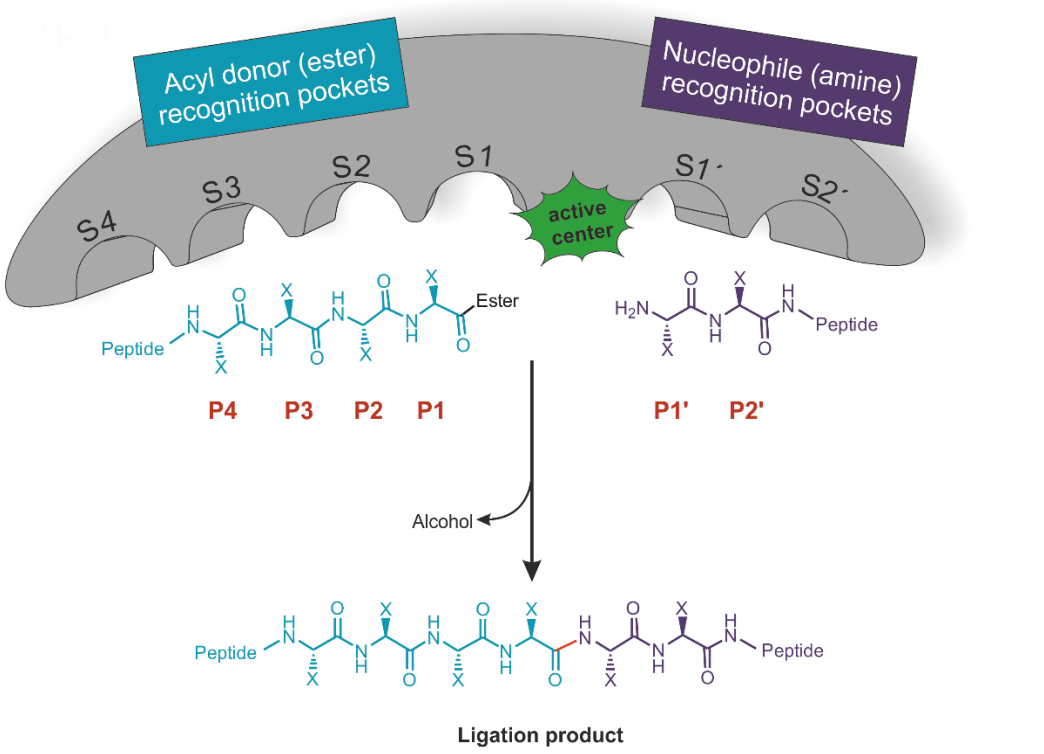
Appendix
Figure 4.
Substrate recognition sites of Omniligase-1

- S1-S4 are substrate pockets at the acyl donor site.
- At the acyl acceptor side, S1′ and S2′ substrate binding pockets are present.



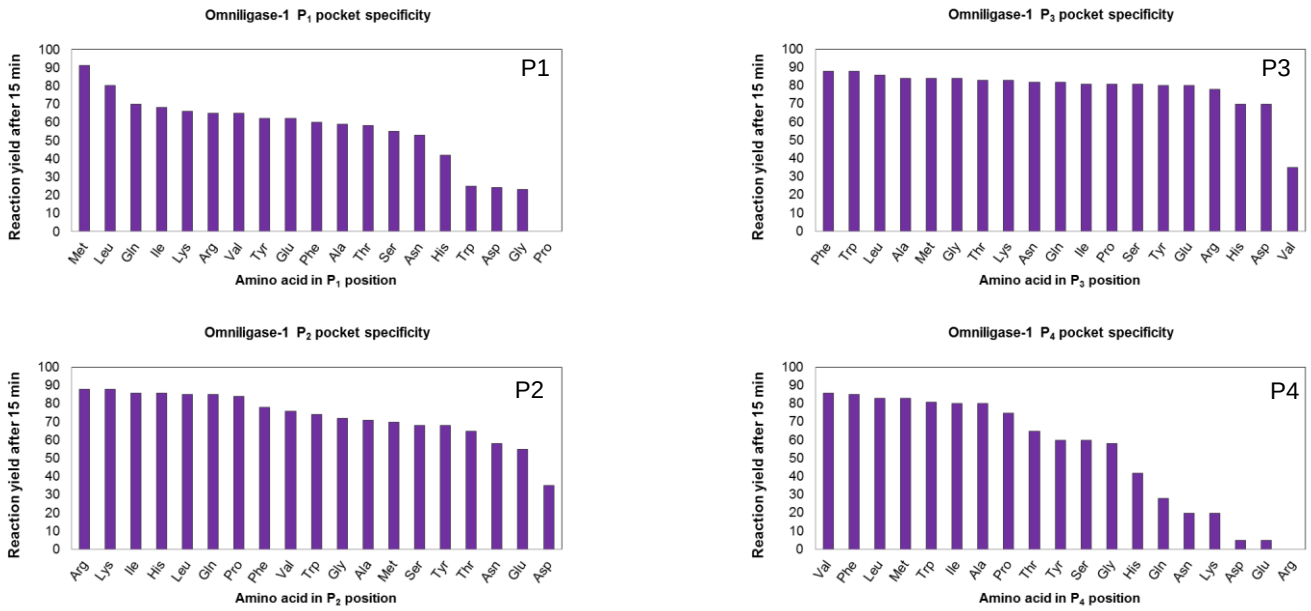
Y-$(Z)_m$-P4-P3-P2-P1-Ester	+	H_2N-P1′′-P2′′-$(Z)_n$-OH (or -NH_2 or Y)
Acyl donor (ester) fragment	+	Nucleophilic (amine) fragment

The fragments are water-soluble, and:

- Z = unprotected or protected amino acid or non-peptidic moiety
- Y= terminal protecting group (optional)
- P4 = hydrophobic L-amino acid or protected L-amino acid
- P3 = any L-amino acid
- P2 = any L-amino acid (preferably not Glu, Asp and Trp)
- P1 = any L-amino acid except Pro (preferably not Gly and Asp)
- P1′ and P2′ = any L-amino acid except Pro
- m, n = any number

Apart from P4-P1, P1′ and P2′, all other components (X, Y, Z) can be of a non-peptide nature, e.g. polymeric.

Figure 5.
Product yield pocket preference profiles of Omniligase-1.



- The P1-P4, P1′ and P2′ pocket preferences are depicted in Figure 5.
- The acyl donor pockets S4-S1 determine the reaction rate. (The product yield after 15 minutes is depicted in Figure 5.).
- The amine pockets S1′ and S2′ determine the reaction yield.
- The S4 pocket prefers hydrophobic P4 amino acids. If appropriate, hydrophobicity of P4 can be achieved by using side-chain protection.

Figure 6.
Heat map substrate profile of Omniligase-1

Amino acid in P1' position (Xxx) \ Amino acid in P2' position (Yyy)	Tyr	Trp	Met	Phe	Ile	Leu	His	Val	Cys	Thr	Gln	Arg	Ala	Lys	Gly	Ser	Asn	Glu	Asp	Pro
Cys	99,5	99,2	99,5	94,6	96,8	94,1	93,2	91,2	92,0	88,3	78,4	89,8	84,2	81,9	84,2	75,2	71,5	58,1	32,4	0,0
Trp	90,9	96,8	89,6	85,4	91,0	82,4	86,2	84,3	77,7	85,7	85,6	88,1	88,3	81,9	80,0	70,7	78,1	60,3	23,3	0,0
Met	98,7	98,3	89,5	90,5	95,6	97,0	95,1	94,5	93,2	89,3	83,6	93,7	63,8	80,6	60,1	59,1	56,6	50,4	18,5	0,0
Phe	98,9	97,2	97,7	95,2	96,2	95,6	83,0	92,0	80,4	73,5	77,5	83,8	71,7	68,8	60,2	46,3	51,1	39,0	13,3	0,0
Ala	92,6	94,4	94,4	76,4	92,0	88,7	83,6	95,2	71,3	88,3	86,1	57,8	66,8	77,3	60,2	35,5	44,8	48,0	10,5	0,0
Gly	85,9	91,1	89,8	78,3	82,6	74,2	76,0	94,7	67,2	60,6	75,7	64,5	60,4	68,1	50,6	58,0	50,2	54,8	13,5	0,0
Ile	87,6	86,0	75,6	93,7	81,6	85,4	93,0	90,6	70,0	46,3	51,1	64,8	91,5	68,3	85,2	39,1	61,1	34,5	11,3	0,0
Ser	90,5	91,0	82,1	71,8	81,2	69,2	77,5	63,6	61,8	69,7	76,5	74,2	72,5	66,6	65,9	39,6	57,7	45,6	9,5	0,0
Val	79,8	92,4	91,1	92,1	79,9	81,9	91,5	66,8	71,6	41,1	59,0	67,0	82,0	60,7	75,9	39,1	59,6	35,5	9,0	0,0
Leu	97,1	98,4	91,8	95,3	71,8	77,0	83,7	78,8	80,0	47,0	62,3	56,9	48,6	52,2	45,3	53,1	40,7	43,3	13,9	0,0
Gln	96,2	79,8	92,0	86,5	84,8	92,0	85,2	93,6	68,5	67,4	45,7	54,4	52,4	49,5	48,3	40,7	32,7	26,0	10,3	0,0
Tyr	94,6	98,3	89,0	91,0	81,0	88,8	84,8	74,7	54,0	64,8	49,5	32,8	38,4	49,8	35,4	31,4	25,4	22,6	7,9	0,0
Arg	94,0	77,8	82,3	81,7	80,2	81,4	70,1	89,7	64,5	52,9	43,9	38,2	42,1	59,5	35,6	24,4	22,1	15,6	14,0	0,0
Lys	92,9	68,3	85,7	89,0	67,9	68,1	74,6	71,0	60,4	43,2	38,7	37,3	37,2	31,6	33,3	25,4	18,0	9,8	7,4	0,0
Asn	90,0	85,5	80,3	77,5	67,7	67,1	72,5	73,4	59,3	43,9	45,9	19,6	45,2	30,8	41,7	14,0	15,1	16,4	4,8	0,0
Thr	84,1	62,5	77,6	63,8	79,0	65,2	74,2	66,8	63,4	48,1	46,7	53,7	31,3	35,3	24,2	18,3	13,7	15,5	5,3	0,0
Asp	89,3	80,0	77,8	79,5	77,1	70,0	63,4	56,2	48,2	19,7	22,5	29,5	22,9	26,7	20,9	11,7	8,2	9,1	3,7	0,0
Glu	93,5	78,5	67,3	75,8	62,9	85,1	57,3	73,1	54,5	31,3	27,4	18,5	21,3	24,5	15,6	13,9	0,0	11,1	2,1	0,0
His	67,3	69,5	60,7	74,3	40,4	43,8	58,6	55,5	46,1	36,3	22,9	29,9	21,9	20,2	18,9	21,0	9,2	12,6	5,1	0,0
Pro	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Omniligase-1 accepts all amino acids, except proline, in the S1′ and S2′ substrate binding pockets.

For the acyl acceptor site, the S1′ and S2′ pockets were screened. All combinations of the first two amino acids in the substrate positions P1′ and P2′ (H-Xxx-Yyy-Peptide) were tested. The yields are reported in the Figure 6 heat map.

The yields shown in the heat map were obtained using 15 mM of acyl acceptor. Higher yields are obtained when the reactions are performed using more concentrated substrate solutions. For obtaining maximal yield of the reaction, it is strongly advised to consult Figure 6 when selecting the ligation positions.