CLONING AND EXPRESSION

DNA Modifying Enzymes

Sigma-Aldrich offers an extensive line of enzymes for molecular biology. Here we provide product information regarding T4 DNA Ligase and restriction endonucleases, which are key enzymes used in molecular cloning experiments.

DNA Ligase from T4-infected *Escherichia coli*

T4 DNA ligase is used for ligation of blunt- or cohesive-ended DNA fragments, for sealing nicks in double stranded DNA and RNA or DNA/RNA hybrids, and for ligation of oligonucleotide adapters.

T4 DNA Ligase produced in *Escherichia coli* is supplied as a solution in 10 mM Tris-HCl, pH 7.5, 50 mM KCl, 1 mM DTT, 50% glycerol (v/v) at 500-6,000 units/ml.

Unit definition: One unit ligates 1 µg of lambda DNA-*Eco*RI fragments in 1 hr at 22 °C in a 20 µl reaction volume and is approximately equal to one Weiss unit.

Product Code	Description	Size
D 2886	DNA Ligase from T4-infected *Escherichia coli*	100 Units 500 Units

DNA Ligation Kit

This kit contains all of the reagents necessary to perform DNA ligation reactions, including polyethylene glycol (PEG 8000), which facilitates blunt-end ligations.

1 kit is sufficient for 150 reactions.

Product Code	Description	Size
LIG-1	DNA Ligation Kit	1 kit

Components:
T4 DNA Ligase
10x Ligation Buffer
24% PEG 8000
10 mM ATP
pBR322, *Hae* III digest (Control)
Water

Quick-Link™ DNA Ligation Kit

Quick-Link has been optimized for efficient blunt- and cohesive-end ligations performed at room temperature with a short incubation, replacing the previous methods requiring 16 °C and long incubations. Pre-made buffers are provided to suit common buffer conditions of the DNA, for simple set-up of reactions.

1 kit is sufficient for 50 reactions.

Features & Benefits

- Rapid 5 minute ligation
- Optimized for ligation at room temperature
- High ligation efficiency
- For blunt- or cohesive-ends

Product Code	Description	Size
LIG-2	Quick-Link™ DNA Ligation Kit	1 kit

Components:
T4 DNA Ligase
2x Ligation Buffer A
5x Ligation Buffer B

Restriction Endonucleases

Sigma offers a variety of restriction endonucleases for cleavage of double-stranded DNA at site-specific sequences. In order to ensure quality, our enzymes are assayed for activity (units/µl), cutting-ligation-recutting, and absence of non-specific endonuclease and exonuclease activity. For complete listings, package sizes and unit concentrations, please refer to the Sigma General Catalog, Life Science Catalog, or the Sigma-Aldrich website.

Restriction endonucleases offered by Sigma-Aldrich are provided in 50% glycerol solutions. We recommend that the glycerol content of reaction mixtures not exceed 5% (v/v).

Restriction Endonuclease Table:

The following table provides information regarding all of our restriction endonucleases such as recognition sequence, incubation temperatures, and percent activity in our core buffers. The optimal incubation temperature is shown only when it differs from 37 °C.

Each enzyme is supplied with the appropriate 10x reaction buffer (indicated in bold). *Nde* II is supplied with a custom buffer (Buffer SP). After dilution to 1x, the buffers have the following compositions:

Buffers SA: 33 mM Tris-acetate, pH 7.9 at 37 °C, 10 mM $Mg(OAc)_2$, 66 mM KOAc, 0.5 mM dithiothreitol (DTT)
Buffers SB: 10 mM Tris-HCl, pH 8.0 at 37 °C, 5 mM $MgCl_2$, 100 mM NaCl, 1 mM 2-mercaptoethanol
Buffers SL: 10 mM Tris-HCl, pH 7.5 at 37 °C, 10 mM $MgCl_2$, 1 mM dithioerythritol (DTE)
Buffers SM: 10 mM Tris-HCl, pH 7.5 at 37 °C, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM dithioerythritol (DTE)
Buffers SH: 50 mM Tris-HCl, pH 7.5 at 37 °C, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM dithioerythritol (DTE)
Buffers SP: 100 mM Tris-HCl, pH 7.6 at 37 °C, 10 mM $MgCl_2$, 150 mM NaCl, 1 mM dithiothreitol (DTT)

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Restriction Endonucleases

Product No.	Enzyme	Recognition Sequence	Isoschizomer	Incubation temp.*	% Activity in 1x buffer				
					SA	SB	SL	SM	SH
R6142	*Acc*I	GT↓(A,C)(T,G)AC			**100**	0-10	10-25	0-10	0-10
R1259	*Afl* III	A↓C(A,G)(T,C)GT			50-75	75-100	50-75	75-100	**100**
R6885	*Alu* I	AG↓CT			**100**	50-75	25-50	25-50	0-10
R4258	*Apa* I	GGGCC↓C		30 °C	**100**	10-25	50-75	50-75	0-10
R3379	*Ava* I	C↓(T,C)CG(A,G)G	*Afl* I		100	**100**	10-25	50-75	10-25
R6004	*Ava* II	G↓G(A,T)CC			**100**	50-75	75-100	100	10-25
R0260	*Bam*H I	G↓GATCC			100	**100**	75-100	100	25-50
R8631	*Bcl* I	T↓GATCA		50 °C	100	100	25-50	**100**	100
R6753	*Bgl* I	GCCN$_4$↓NGGC			25-50	50-75	10-25	25-50	**100**
R6377	*Bgl* II	A↓GATCT			100	100	25-50	**100**	100
R3131	*Bln* I	C↓CTAGG	*Avr* II		25-50	50-75	0-10	25-50	**100**
R3635	*Bsm* I	GAATGCN↓N		65 °C	0-10	50-75	0-10	25-50	**100**
R2634	*Bss*H II	G↓CGCGC		50 °C	**100**	100	75-100	100	75-100
R4253	*Bst*E II	G↓GTNACC		60 °C	75-100	**100**	25-50	50-75	50-75
R1761	*Cfo* I	GCG↓C	*Hha* I		75-100	50-75	**100**	50-75	25-50
R7763	*Cla* I	AT↓CGAT			100	100	75-100	100	**100**
R4256	*Dde* I	C↓TNAG			50-75	75-100	25-50	25-50	**100**
R8381	*Dpn* I	G^{m}A↓TC			**100**	75-100	50-75	75-100	75-100
R4381	*Dra* I	TTT↓AAA	*Aha* III		100	75-100	100	**100**	50-75
R3884	*Ecl*X I	C↓GGCCG	*Xma* III		25-50	**100**	25-50	25-50	50-75
R6265/R4640	*Eco*R I	G↓AATTC			100	100	25-50	50-75	**100**
R1636	*Eco*R II	↓CC(A,T)GG			50-75	75-100	0-25	50-75	**100**
R2756	*Eco*R V	GAT↓ATC			25-50	**100**	0-10	25-50	50-75
R4257	*Hae* II	(A,G)GCGC↓(T,C)			**100**	50-75	25-50	50-75	10-25
R5628	*Hae* III	GG↓CC			50-75	50-75	75-100	**100**	25-50
R1382	*Hind* II	GT(T,C)↓(A,G)AC	*Hinc* II		100	100	25-50	**100**	50-75
R1137	*Hind* III	A↓AGCTT			50-75	**100**	25-50	100	50-75
R8507	*Hpa* I	GTT↓AAC			**100**	25-50	25-50	50-75	25-50
R0629	*Hpa* II	C↓CGG	*Msp* I		50-75	25-50	**100**	50-75	10-25
R1258	*Kpn* I	GGTAC↓C			75-100	10-25	**100**	25-50	0-10
R4134	*Ksp* I	CCGC↓GG	*Sac* II		0-10	0-10	**100**	0-10	0-10
R8257	*Mlu* I	A↓CGCGT			10-25	25-50	0-10	10-25	**100**
R1507	*Mlu* NI	TGG↓CCA	*Bal* I		**100**	0-10	10-25	10-25	0-10
R4506	*Msp* I	C↓CGG			100	100	**100**	100	50-75
R1632	*Mva* I	CC↓(A,T)GG	*Bst*N I		100	50-75	25-50	25-50	**100**
R5635	*Nci* I	CC↓(G,C)GG			75-100	50-75	**100**	10-25	0-10
R8761	*Nco* I	C↓CATGG			50-75	50-75	50-75	50-75	**100**
R5509	*Nde* I	CA↓TATG			25-50	100	10-25	50-75	**100**
R5757	*Nde* II*	↓GATC	*Mbo* I		10-25	10-25	0-10	0-10	10-25
R5634	*Nhe* I	G↓CTAGC			100	25-50	100	**100**	10-25
R8506	*Not* I	GC↓GGCCGC			10-25	50-75	0-10	25-50	**100**
R5884	*Nsi* I	ATGCA↓T	*Ava* III		50-75	100	10-25	50-75	**100**
R7023	*Pst* I	CTGCA↓G			25-50	25-50	10-25	25-50	**100**
R1508	*Pvu* I	CGAT↓CG			50-75	75-100	25-50	50-75	**100**
R2631	*Pvu* II	CAG↓CTG			25-50	25-50	25-50	**100**	25-50
R4756	*Rsa* I	GT↓AC			100	50-75	**100**	50-75	0-10
R5268	*Sac* I	GAGCT↓C	*Sst* I		**100**	0-10	100	50-75	0-10
R0754	*Sal* I	G↓TCGAC			0-10	25-50	0-10	10-25	**100**
R0762	*Sau*3A I	↓GATC			**100**	25-50	25-50	75-100	0-10
R5007	*Sca* I	AGT↓ACT			0-10	100	0-10	75-100	**100**
R7888	*Scr* FI	CC↓NGG	*Dsa* V		10-25	**100**	10-25	10-25	50-75
R8256	*Sfi* I	GGCCN$_4$↓NGGCC		50 °C	25-50	25-50	75-100	**100**	25-50
R4503	*Sma* I	CCC↓GGG		25 °C	**100**	0-10	0-10	0-10	0-10
R5257	*Spe* I	A↓CTAGT			75-100	75-100	75-100	100	**100**
R7135	*Sph* I	GCATG↓C			50-75	75-100	25-50	**100**	75-100
R6759	*Ssp* I	AAT↓ATT			75-100	75-100	10-25	75-100	**100**
R8013	*Stu* I	AGG↓CCT	*Aat* I		100	**100**	100	100	50-75
R7009	*Sty* I	C↓C(A,T)(A,T)GG			50-75	100	10-25	75-100	**100**
R1885	*Swa* I	ATTT↓AAAT		25 °C	0-10	10-25	0-10	0-10	**100**
R9507	*Taq* I	T↓CGA		65 °C	50-75	**100**	25-50	50-75	50-75
R7260	*Xba* I	T↓CTAGA			100	75-100	75-100	75-100	**100**
R6379	*Xho* I	C↓TCGAG			25-50	75-100	10-25	25-50	**100**