Thermo Scientific Conventional
Restriction Enzymes Buffer Activity Chart

Enzyme properties codes used below: ★ = enzyme is prone to star activity; 65 / 80 = thermal inactivation at 65 °C / 80 °C (20 min); No = no thermal inactivation; 80 (≤10 u) = up to 10 units of enzyme can be inactivated at 80°C in 20 min; Dam, Dcm, CG = methylation sensitivity.

Enzyme	Specificity 5'→3'	Recommended buffer for 100% activity	B (blue) 1X	G (green) 1X	O (orange) 1X	R (red) 1X	Tango™ (yellow) 1X	Tango™ (yellow) 2X	Thermo Scientific™ Tango™ buffer for double digestion	Enzyme properties
AanI (PsiI)	TTA↓TAA	Tango	50-100	50-100	0-20	0-20	100	20-50	1X or 2X	65
AarI	CACCTGC(4/8)↓	Unique (+oligo)	NR	NR	0-20 (+oligo)	0-20 (+oligo)	NR	50-100 (+oligo)	2X (+oligo)	★ 65 CG
AatII	GACGT↓C	Tango	50-100	20-50	0-20	0-20	100	20-50	1X or 2X	65 CG
Acc65I (Asp718I)	G↓GTACC	O	0-20	20-50	100	20-50	20-50	50-100	1X or 2X	65 Dcm CG
AdeI (DraIII)	CACNNN↓GTG	G	0-20	100	20-50	100	100*	20-50	1X* or 2X	★ No CG
AjiI (BmgBI)	CAC↓GTC	Unique	NR	NR	20-50*	NR	NR	20-50*	2X*	65 CG
AjuI	↓(7/12)GAA(N)$_7$TTGG(11/6)↓	R (+SAM)	0-20 (+SAM)	50-100 (+SAM)	20-50 (+SAM)	100 (+SAM)	50-100 (+SAM)	50-100 (+SAM)	1X or 2X (+SAM)	65
AlfI	↓(10/12)GCA(N)$_6$TGC(12/10)↓	R (+SAM)	0-20 (+SAM)	0-20 (+SAM)	0-20 (+SAM)	100 (+SAM)	0-20 (+SAM)	20-50 (+SAM)	2X (+SAM)	65
AloI (30°C)	↓(7/12-13)GAAC(N)$_6$TCC(12-13/7)↓	R	0-20	0-20	0-20	100	20-50	100	1X or 2X	★ 65 CG
AluI	AG↓CT	Tango	50-100	0-20	0-20	0-20	100	20-50	1X or 2X	65
Alw21I (BsiHKAI)	GWGCW↓C	O	0-20	20-50	100	50-100	20-50	50-100	1X or 2X	65
Alw26I (BsmAI)	GTCTC(1/5)↓	Tango	50-100	100	0-20	0-20	100	100	1X or 2X	65 CG
Alw44I (ApaLI)	G↓TGCAC	Tango	50-100	100	0-20	50-100	100	50-100	1X or 2X	65 CG
ApaI	GGGCC↓C	B	100	20-50	0-20	0-20	20-50	0-20	1X	65 Dcm CG
BamHI	G↓GATCC	Unique	20-50*	100	20-50	50-100*	100*	50-100	1X* or 2X	80 (≤10 u)
BauI (BssSI)	CACGAG(-5/-1)↓	Tango	0-20	50-100	0-20	50-100	100	50-100	1X or 2X	65
BclI (55°C)	T↓GATCA	G	20-50	100	20-50	20-50	100*	100	1X* or 2X	★ 80 (≤10 u) Dam
BcnI (NciI)	CC↓SGG	Tango	20-50	50-100	50-100	50-100	100	50-100	1X or 2X	65 CG
BcuI (SpeI)	A↓CTAGT	Tango	50-100	50-100	0-20	20-50	100	0-20	1X	No
BfmI (SfcI)	C↓TRYAG	Tango	0-20	50-100	0-20	0-20	100	20-50	1X or 2X	65
BfuI (BciVI)	GTATCC(6/5)↓	Unique	NR	NR	0-20	0-20	NR	50-100*	2X*	★ 80
BglI	GCCNNNN↓NGGC	O	0-20	50-100	100	100	0-20	100	2X	65 CG
BglII	A↓GATCT	O	0-20	20-50	100	50-100	0-20	100	2X	No
Bme1390I (ScrFI)	CC↓NGG	O	20-50	50-100	100	50-100	50-100	50-100	1X or 2X	80 Dcm CG
BoxI (PshAI)	GACNN↓NNGTC	Tango	0-20	0-20	0-20	20-50	100	20-50	1X or 2X	80 CG
BpiI (BbsI)	GAAGAC(2/6)↓	G	20-50	100	50-100	50-100	50-100	50-100	1X or 2X	65
BplI	↓(8/13)GAG(N)$_5$CTC(13/8)↓	Tango (+SAM)	0-20 (+SAM)	20-50 (+SAM)	0-20 (+SAM)	0-20 (+SAM)	100 (+SAM)	20-50 (+SAM)	1X (+SAM)	65
Bpu10I	CCTNAGC(-5/-2)↓	Unique	0-20	20-50*	50-100*	100*	50-100*	100*	1X* or 2X*	80
Bpu1102I (BlpI)	GC↓TNAGC	Tango	50-100	50-100	20-50	20-50	100	20-50	1X or 2X	80
BseDI (BsaJI) (55°C)	C↓CNNGG	Tango	50-100	20-50	0-20	0-20	100	50-100	1X or 2X	80
BseGI (BtsCI) (55°C)	GGATG(2/0)↓	Tango	20-50	50-100	20-50	20-50	100	20-50	1X or 2X	80
BseJI (BsaBI) (65°C)	GATNN↓NNATC	O	NR	100*	100	NR	NR	100*	2X*	★ No Dam
BseLI (BslI) (55°C)	CCNNNNN↓NNGG	Tango	20-50	100	50-100	20-50	100	50-100	1X or 2X	No Dcm CG
BseMI (BsrDI) (55°C)	GCAATG(2/0)↓	R	0-20	20-50	0-20	100	50-100	50-100	1X or 2X	80
BseMII (BspCNI) (55°C)	CTCAG(10/8)↓	Tango (+SAM)	50-100 (+SAM)	50-100 (+SAM)	50-100 (+SAM)	50-100 (+SAM)	100 (+SAM)	50-100 (+SAM)	1X or 2X (+SAM)	80
BseNI (BsrI) (65°C)	ACTGG(1/-1)↓	B	100	20-50	0-20	0-20	50-100	20-50	1X or 2X	80
BseSI (Bme1580I) (55°C)	GKGCM↓C	G	20-50	100	0-20	20-50	50-100	0-20	1X	80 (≤10 u)
BseXI (BbvI) (65°C)	GCAGC(8/12)↓	Unique	NR	NR	NR	NR	NR	NR	NR	★ 80
Bsh1236I (BstUI)	CG↓CG	R	0-20	0-20	50-100	100	20-50	50-100	1X or 2X	65 CG
Bsh1285I (BsiEI)	CGRY↓CG	G	20-50	100	20-50	0-20	0-20	20-50	2X	80 Dam CG
BshNI (BanI)	G↓GYRCC	O	0-20	20-50	100	50-100	0-20	100	2X	65 Dcm CG
BshTI (AgeI)	A↓CCGGT	O	0-20	20-50	100	50-100	20-50	20-50	1X or 2X	80 CG
Bsp68I (NruI)	TCG↓CGA	O	0-20	20-50	100	50-100	20-50	50-100	1X or 2X	★ 65 CG
Bsp119I (BstBI)	TT↓CGAA	Tango	20-50	0-20	0-20	0-20	100	100	1X or 2X	80 CG
Bsp120I (PspOMI)	G↓GGCCC	B	100	20-50	0-20	20-50	50-100	0-20	1X	80 Dcm CG
Bsp143I (Sau3AI)	↓GATC	Unique	20-50	20-50	0-20	0-20	50-100	20-50	1X or 2X	65 CG
Bsp1407I (BsrGI)	T↓GTACA	Tango	0-20	20-50	0-20	20-50	100	50-100	1X or 2X	65
BspLI (NlaIV)	GGN↓NCC	Tango	50-100	50-100	0-20	20-50	100	20-50	1X or 2X	65 Dcm CG
BspOI (BmtI)	GCTAG↓C	O	0-20	0-20	100	100	0-20	20-50	2X	80
BspPI (AlwI) (55°C)	GGATC(4/5)↓	Tango	20-50	20-50	0-20	0-20	100	0-20	1X	80 Dam
BspTI (AflII)	C↓TTAAG	O	0-20	0-20	100	20-50	0-20	50-100	2X	65
Bst1107I (BstZ17I)	GTA↓TAC	O	20-50	50-100	100	100	20-50	100	1X or 2X	80 CG
BstXI (55°C)	CCANNNNN↓NTGG	O	20-50	100	100	50-100	50-100	100	1X or 2X	★ 80 Dcm
Bsu15I (ClaI)	AT↓CGAT	Tango	20-50	20-50	20-50	20-50	100	20-50	1X or 2X	65 Dam CG
BsuRI (HaeIII)	GG↓CC	R	20-50	20-50	50-100	100	50-100	100	1X or 2X	80
BveI (BspMI)	ACCTGC(4/8)↓	O (+oligo)	0-20 (+oligo)	20-50 (+oligo)	100 (+oligo)	20-50 (+oligo)	50-100 (+oligo)	100 (+oligo)	1X or 2X (+oligo)	65 CG
CaiI (AlwNI)	CAGNNN↓CTG	Tango	20-50	20-50	20-50	50-100	100	50-100	1X or 2X	65 Dcm
Cfr9I (XmaI)	C↓CCGGG	Unique	0-20	0-20	0-20	0-20	20-50	0-20	1X	65 CG
Cfr10I (BsrFI)	R↓CCGGY	Unique	0-20	20-50	20-50	50-100*	20-50	50-100	1X or 2X	★ No CG
Cfr13I (Sau96I)	G↓GNCC	Tango	50-100	50-100	20-50	20-50	100	20-50	1X or 2X	65 Dcm CG
Cfr42I (SacII)	CCGC↓GG	B	100	50-100	0-20	0-20	50-100	0-20	1X	65 CG
CpoI (RsrII)	CG↓GWCCG	Tango	20-50	50-100	50-100	20-50	100	50-100	1X or 2X	65 CG
CseI (HgaI)	GACGC(5/10)↓	R	NR	50-100*	50-100	100	100*	50-100	1X* or 2X	★ 80 (≤10 u) CG
Csp6I (CviQI)	G↓TAC	B	100	50-100	0-20	0-20	50-100	0-20	1X	65
DpnI	Gm6A↓TC	Tango	100	100	50-100	50-100	100	50-100	1X or 2X	80
DraI	TTT↓AAA	Tango	50-100	50-100	20-50	20-50	100	50-100	1X or 2X	65
Eam1104I (EarI)	CTCTTC(1/4)↓	Tango	50-100	50-100	0-20	0-20	100	0-20	1X	65
Eam1105I (AhdI)	GACNNN↓NNGTC	Unique	20-50	50-100	0-20	0-20	50-100	20-50	1X or 2X	65
Ecl136II (EcoICRI)	GAG↓CTC	Unique	50-100	20-50	0-20	0-20	50-100	0-20	1X	65 CG
Eco24I (BanII)	GRGCY↓C	Tango	50-100	50-100	0-20	20-50	100	0-20	1X	65
Eco31I (BsaI)	GGTCTC(1/5)↓	G	50-100	100	0-20	0-20	50-100	20-50	1X or 2X	65 Dcm CG
Eco32I (EcoRV)	GAT↓ATC	R	0-20	50-100	50-100	100	20-50	100	1X or 2X	80
Eco47I (AvaII)	G↓GWCC	R	0-20	50-100	50-100	100	50-100	50-100	1X or 2X	65 Dcm CG
Eco47III (AfeI)	AGC↓GCT	O	0-20	20-50	100	100	50-100	100	1X or 2X	65 CG
Eco52I (EagI)	C↓GGCCG	Unique	0-20	0-20	0-20	20-50	0-20	20-50	2X	65 CG
Eco57I (AcuI)	CTGAAG(16/14)↓	G (+SAM)	100 (+SAM)	100 (+SAM)	20-50 (+SAM)	20-50 (+SAM)	50-100 (+SAM)	50-100 (+SAM)	1X or 2X (+SAM)	65
Eco72I (PmlI)	CAC↓GTG	Tango	NR	NR	0-20	0-20	100	20-50	1X or 2X	★ 65 CG
Eco81I (Bsu36I)	CC↓TNAGG	Tango	50-100	100	0-20	0-20	100	0-20	1X	80
Eco88I (AvaI)	C↓YCGRG	Tango	100	50-100	0-20	0-20	100	20-50	1X or 2X	65 CG
Eco91I (BstEII)	G↓GTNACC	O	20-50	20-50	100	50-100	NR	100	1X or 2X	65
Eco105I (SnaBI)	TAC↓GTA	Tango	100*	50-100	0-20	0-20	100	0-20	1X	★ 65 CG
Eco130I (StyI)	C↓CWWGG	O	0-20	20-50	100	50-100	50-100	100	1X or 2X	65
Eco147I (StuI)	AGG↓CCT	B	100	50-100	20-50	20-50	50-100	0-20	1X	80 Dcm
EcoO109I (DraII)	RG↓GNCCY	Tango	50-100	20-50	20-50	20-50	100	100	1X or 2X	65 Dcm
EcoRI	G↓AATTC	Unique	0-20	NR	100	100*	NR	100	2X	65 CG
EcoRII	↓CCWGG	O	20-50	50-100	100	50-100	20-50	50-100	1X or 2X	80 Dcm
EheI (SfoI)	GGC↓GCC	Tango	20-50	50-100	0-20	0-20	100	20-50	1X or 2X	65 CG
Esp3I (BsmBI)	CGTCTC(1/5)↓	Tango (+DTT)	100 (+DTT)	20-50 (+DTT)	0-20 (+DTT)	0-20 (+DTT)	100 (+DTT)	0-20 (+DTT)	1X (+DTT)	65 CG
FaqI (BsmFI)	GGGAC(10/14)↓	Tango (+SAM)	20-50 (+SAM)	20-50 (+SAM)	0-20 (+SAM)	0-20 (+SAM)	100 (+SAM)	20-50 (+SAM)	1X or 2X (+SAM)	80 CG
FspAI	RTGC↓GCAY	O	0-20	0-20	100	50-100	0-20	50-100	2X	65 CG
FspBI (BfaI)	C↓TAG	Tango	50-100	20-50	0-20	0-20	100	0-20	1X	65
GsuI (BpmI) (30°C)	CTGGAG(16/14)↓	B	100	50-100	20-50	20-50	100	50-100	1X or 2X	65 Dcm
HhaI	GCG↓C	Tango	50-100	50-100	20-50	20-50	100	20-50	1X or 2X	No CG
Hin1I (BsaHI)	GR↓CGYC	G	20-50	100	20-50	20-50	20-50	20-50	1X or 2X	65 Dcm CG
Hin1II (NlaIII)	CATG↓	G	50-100	100	20-50	50-100	50-100	50-100	1X or 2X	65
Hin6I (HinP1I)	G↓CGC	Tango	50-100	50-100	20-50	20-50	100	50-100	1X or 2X	65 CG
HincII (HindII)	GTY↓RAC	Tango	50-100	50-100	20-50	50-100	100	50-100	1X or 2X	65 CG
HindIII	A↓AGCTT	R	0-20	20-50	0-20	100	50-100	50-100	1X or 2X	80
HinfI	G↓ANTC	R	0-20	20-50	50-100	100	50-100	50-100	1X or 2X	65 CG
HpaII	C↓CGG	Tango	50-100	50-100	0-20	20-50	100	20-50	1X or 2X	65 CG
HphI	GGTGA(8/7)↓	B	100	0-20	0-20	0-20	20-50	0-20	1X	65 Dam
Hpy8I (MjaIV)	GTN↓NAC	Tango	50-100	50-100	0-20	20-50	100	50-100	1X or 2X	80 CG
HpyF3I (DdeI)	C↓TNAG	Tango	20-50	20-50	20-50	20-50	100	50-100	1X or 2X	65
HpyF10VI (MwoI)	GCNNNNN↓NNGC	Tango	0-20	0-20	0-20	0-20	100	50-100	1X or 2X	80 CG
KpnI	GGTAC↓C	Unique	20-50	0-20	0-20	0-20	20-50	0-20	1X	80
Kpn2I (BspEI) (55°C)	T↓CCGGA	Tango	50-100	50-100	0-20	20-50	100	50-100	1X or 2X	80 CG
KspAI (HpaI)	GTT↓AAC	B	100	50-100*	20-50	20-50	100*	50-100	1X* or 2X	65 CG
LguI (SapI)	GCTCTTC(1/4)↓	Tango	20-50	50-100	20-50	20-50	100	20-50	1X or 2X	65
Lsp1109I (BbvI)	GCAGC(8/12)↓	Unique	0-20	20-50*	50-100*	100*	20-50*	20-50*	1X* or 2X*	★ 65
LweI (SfaNI)	GCATC(5/9)↓	Tango	0-20	0-20	0-20	20-50	100	20-50	1X or 2X	65 CG
MauBI	CG↓CGCGCG	Tango	0-20	0-20	0-20	0-20	100	0-20	1X	65 CG
MbiI (BsrBI)	CCGCTC(-3/-3)↓	Tango	20-50	100	20-50	20-50	100	20-50	1X or 2X	65 CG
MboI	↓GATC	R	50-100	50-100	50-100	100	50-100	100	1X or 2X	65 Dam
MboII	GAAGA(8/7)↓	B	100	50-100	20-50	0-20	50-100	20-50	1X or 2X	★ 65 Dam
MlsI (MscI)	TGG↓CCA	R	0-20	20-50	0-20	100	20-50	50-100	1X or 2X	65 Dcm
MluI	A↓CGCGT	R	0-20	20-50	50-100	100	20-50	50-100	1X or 2X	80 CG
MnlI	CCTC(7/6)↓	G	50-100	100	20-50	20-50	20-50	20-50	1X or 2X	65
Mph1103I (NsiI)	ATGCA↓T	R	0-20	50-100	20-50	100	50-100	50-100	1X or 2X	65
MreI (Sse232I)	CG↓CCGGCG	G	20-50	100	0-20	0-20	50-100	0-20	1X	80 CG
MspI (HpaII)	C↓CGG	Tango	50-100	50-100	0-20	0-20	100	50-100	1X or 2X	80
MssI (PmeI)	GTTT↓AAAC	B	100	0-20	0-20	0-20	20-50	0-20	1X	65
MunI (MfeI)	C↓AATTG	G	100	100	0-20	0-20	100	0-20	1X	65
MvaI (BstNI)	CC↓WGG	R	20-50	20-50	50-100	100	20-50*	100	1X* or 2X	No
Mva1269I (BsmI)	GAATGC(1/-1)↓	R	0-20	20-50	50-100	100	0-20	50-100	2X	65
NcoI	C↓CATGG	Tango	20-50	20-50	20-50	50-100	100	100	1X or 2X	65
NdeI	CA↓TATG	O	0-20	0-20	100	50-100	0-20	50-100	2X	65
NheI	G↓CTAGC	Tango	100	20-50	0-20	0-20	100	0-20	1X	65 CG
NmuCI (Tsp45I)	↓GTSAC	R	0-20	20-50	50-100	100	20-50	50-100	1X or 2X	65 CG
NotI	GC↓GGCCGC	O	0-20	0-20	100	20-50	0-20	20-50	2X	80 CG
NsbI (FspI)	TGC↓GCA	Tango™	20-50	50-100	0-20	20-50	100	20-50	1X or 2X	65 CG
OliI (AleI)	CACNN↓NNGTG	R	0-20	0-20	0-20	100	0-20	50-100	2X	65 CG
PacI	TTAAT↓TAA	Unique	20-50	20-50	0-20	0-20	0-20	0-20	NR	65
PaeI (SphI)	GCATG↓C	B	100	50-100	0-20	0-20	50-100	0-20	1X	65
PagI (BspHI)	T↓CATGA	O	0-20	50-100	100	NR	NR	NR	NR	80 Dam
PasI (55°C)	CC↓CWGGG	Unique	NR	NR	NR	NR	NR	NR	NR	★ 80
PauI (BssHII)	G↓CGCGC	R	0-20	0-20	100	100	0-20	100	2X	80 CG
PdiI (NaeI)	GCC↓GGC	Tango	50-100	20-50	0-20	0-20	100	50-100	1X or 2X	65 CG
PdmI (XmnI)	GAANN↓NNTTC	Tango	20-50	50-100	0-20	0-20	100	0-20	1X	65 CG
PfeI (TfiI)	G↓AWTC	O	0-20	20-50	100	50-100	20-50	50-100	1X or 2X	65 CG
Pfl23II (BsiWI)	C↓GTACG	Tango	20-50	50-100	20-50	20-50	100	0-20	1X	65 CG
PfoI	T↓CCNGGA	Tango	0-20	20-50	50-100	0-20	100	50-100	1X or 2X	65 Dam Dcm CG
Ppu21I (BsaAI) (30°C)	YAC↓GTR	Unique	50-100*	100*	20-50	NR	NR	NR	NR	65 CG
PscI (PciI)	A↓CATGT	Tango	20-50	20-50	0-20	0-20	100	0-20	1X	65
Psp5II (PpuMI)	RG↓GWCCY	G	0-20	100	20-50	20-50	50-100	100	1X or 2X	80 Dcm
Psp1406I (AclI)	AA↓CGTT	Tango	100	50-100	0-20	20-50	100	0-20	1X	65 CG
PstI	CTGCA↓G	O	50-100	50-100	100	100	50-100	50-100	1X or 2X	No
PsuI (BstYI)	R↓GATCY	B	100	20-50	0-20	0-20	50-100	0-20	1X	80
PsyI (Tth111I)	GACN↓NNGTC	B	100	50-100	0-20	0-20	50-100	0-20	1X	80
PvuI	CGAT↓CG	R	0-20	20-50	50-100	100	50-100	100	1X or 2X	65 CG
PvuII	CAG↓CTG	G	50-100*	100	20-50	50-100	20-50*	20-50*	1X* or 2X*	★ No
RsaI	GT↓AC	Tango	50-100	20-50	0-20	0-20	100	0-20	1X	80 CG
RseI (MslI)	CAYNN↓NNRTG	R	0-20	50-100	50-100	100	20-50	100	1X or 2X	65
SacI	GAGCT↓C	Unique	50-100	20-50	0-20	0-20	50-100	20-50	1X or 2X	65
SalI	G↓TCGAC	O	0-20	0-20	100	20-50	0-20	50-100	2X	65 CG
SatI (Fnu4HI)	GC↓NGC	G	20-50	100	20-50	20-50	50-100	20-50	1X or 2X	65 CG
ScaI	AGT↓ACT	Unique	0-20	0-20	0-20	0-20	0-20	0-20	NR	80 (≤10 u)
SchI (MlyI)	GAGTC(5/5)↓	Tango	20-50	50-100	0-20	0-20	100	0-20	1X	★ 65
SdaI (SbfI)	CCTGCA↓GG	Unique	NR	NR	0-20	0-20	NR	20-50	2X	★ 80
SduI (Bsp1286I)	GDGCH↓C	Unique	NR	50-100*	50-100	0-20	NR	NR	NR	65
SfaAI (AsiSI)	GCGAT↓CGC	Tango	50-100	0-20	0-20	0-20	100	0-20	1X	80 CG
SfiI (50°C)	GGCCNNNN↓NGGCC	G	50-100	100	20-50	0-20	100	0-20	1X	No Dcm CG
SgeI	m5CNNG(9/13)↓	Unique	0-20	0-20	0-20	NR	NR	NR	NR	★ 65
SgrDI	CG↓TCGACG	R	0-20	0-20	0-20	100	NR	100	2X	65 CG
SgsI (AscI)	GG↓CGCGCC	Tango	0-20	0-20	0-20	50-100	100	50-100	1X or 2X	65 CG
SmaI (30°C)	CCC↓GGG	Tango	50-100	0-20	0-20	0-20	100	0-20	1X	65 CG
SmiI (SwaI) (30°C)	ATTT↓AAAT	O	0-20	0-20	100	20-50	0-20	20-50	2X	65
SmoI (SmlI) (55°C)	C↓TYRAG	Tango	50-100	20-50	0-20	20-50	100	20-50	1X or 2X	80 Dam
SsiI (AciI)	CCGC(-3/-1)↓	O	NR	20-50	100	50-100	NR	100	2X	65 CG
SspI (KasI)	AAT↓ATT	G	20-50	100	0-20	50-100	100	20-50	1X or 2X	★ 65
SspDI (KasI)	G↓GCGCC	Tango	20-50	0-20	NR	0-20	100	20-50	1X or 2X	80 CG
TaaI (HpyCH4III) (65°C)	ACN↓GT	Tango	0-20	0-20	0-20	50-100	100	100	1X or 2X	No CG
TaiI (MaeII)neo (65°C)	ACGT↓	R	50-100	50-100	20-50	100	100	50-100	1X or 2X	No CG
TaqI (65°C)	T↓CGA	Unique	0-20	20-50	20-50	20-50	20-50	20-50	1X or 2X	No Dam
TasI (Tsp509I) (65°C)	↓AATT	B	100	50-100	20-50	0-20	20-50	0-20	1X	No
TatI (65°C)	W↓GTACW	Tango	NR	50-100*	20-50	20-50	100*	0-20	1X*	★ No
TauI (55°C)	GCSG↓C	B	100	50-100	0-20	0-20	20-50	0-20	1X	No CG
Tru1I (MseI) (65°C)	T↓TAA	R	50-100	50-100	20-50	100	50-100	100	1X or 2X	No
TscAI (TspRI) (65°C)	CASTG(2/-7)↓	Tango	50-100	50-100	20-50	20-50	100	20-50	1X or 2X	No
Van91I (PflMI)	CCANNNN↓NTGG	R	0-20	50-100	50-100	100	20-50	50-100	1X or 2X	65 Dcm
VspI (AseI)	AT↓TAAT	O	0-20	50-100	100	20-50	100	100	1X or 2X	65
XagI (EcoNI)	CCTNN↓NNNAGG	R	0-20	20-50	50-100	100	20-50	50-100	1X or 2X	65
XapI (ApoI)	R↓AATTY	Tango	50-100	100	0-20	0-20	100	20-50	1X or 2X	★ 80
XbaI	T↓CTAGA	Tango	50-100	50-100	20-50	0-20	100	50-100	1X or 2X	65 Dam
XceI (NspI)	RCATG↓Y	Tango	50-100	0-20	0-20	0-20	100	0-20	1X	65
XhoI	C↓TCGAG	R	0-20	50-100	50-100	100	20-50	100	1X or 2X	80 CG
XmaJI (AvrII)	C↓CTAGG	Tango	20-50	50-100	50-100	50-100	100	50-100	1X or 2X	80
XmiI (AccI)	GT↓MKAC	B	100	0-20	0-20	0-20	50-100	20-50	1X or 2X	65 CG
Nicking Enzymes										
Nb.Bpu10I	GC↓TNAGG	R	0-20	20-50	20-50	100	20-50	50-100	1X or 2X	80
Nt.Bpu10I	CC↓TNAGC	R	0-20	0-20	100	100	0-20	50-100	2X	65
Nb.Mva1269I	G↓CATTC	O	0-20	20-50	100	100	20-50	50-100	1X or 2X	80
Homing Enzyme										
I-SceI	TAGGGATAA↓CAGGGTAAT	Tango	50-100	50-100	50-100	50-100	100	50-100	1X or 2X	65

Buffer Compatibility for Common Modifying Enzymes

Enzyme activity in 1X buffers, %

DNA/RNA Modifying Enzyme	Thermo Scientific™ FastDigest™/ FastDigest™ Green	B	G	O	R	Tango	2X Tango	BamHI	Ecl136II, SacI	EcoRI	KpnI	*Taq* with KCl	*Taq* with $(NH_4)_2SO_4$	Thermo Scientific™ RevertAid RT	T4 DNA Ligase
T4 DNA Ligase*	75-100	100	100	75-100	75-100	75-100	75-100	75-100	50	75-100	100	75	75	75	100
Thermo Scientific™ FastAP™ Thermosensitive Alkaline Phosphatase	100	100	100	100	100	100	100	100	100	100	100	100	50	100	ND
T4 Polynucleotide Kinase** (T4 PNK)	100	75-100	100	100	75-100	100	100	100	50-75	100	75-100	100	0	100	100
DNA Polymerase I	100	25-50	75-100	100	100	100	100	100	50-75	100	50-75	100	100	100	ND
Klenow Fragment	100	25-50	20-50	100	100	100	100	100	50-75	100	50-75	100	100	100	100
Klenow Fragment, exo⁻	100	25-50	25-50	100	100	100	100	100	50-75	100	75-100	100	100	100	ND
T4 DNA Polymerase	100	75-100	75-100	100	100	100	100	100	100	100	100	50	100	100	100
T7 DNA Polymerase	100	75-100	100	100	100	100	100	75-100	75-100	100	100	ND	ND	ND	ND
Exonuclease I (Exo I)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	100	100	ND	ND
Exonuclease III (Exo III)	0-25	100	25-50	0-25	0-25	25-50	0-25	0-25	100	0-25	100	ND	ND	ND	ND
Lambda Exonuclease	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	50-75	50-75	ND	ND
phi29 DNA Polymerase	100	25-100	100	100	100	100	100	100	50-75	100	50-75	25-50	75-100	75-100	25-100
Bsm DNA Polymerase	100	25-50	75-100	100	100	100	100	100	0-25	100	25-50	100	100	100	50-75

* – buffers were supplemented with 0.5 mM ATP, required for T4 DNA Ligase activity, ** – the activity of this enzyme was compared to activity in buffer A (for forward reaction), ND – not determined.

How do I Perform a Double Digest?

Three options to achieve a successful double digest:

1. **Thermo Scientific™ DoubleDigest™ Web Tool**
   - Visit thermoscientific.com/doubledigest to determine optimal reaction conditions for double digests. Simply select two restriction enzymes, submit the query and follow the recommendations.
2. **Double Digestion using Universal Tango Buffer**
   - Determine the concentration of Tango buffer recommended for each restriction enzyme.
   - If the recommended concentration of Tango buffer is the same for both enzymes, the two digests can be performed simultaneously.
   - If the two restriction enzymes require different concentrations of Tango buffer, perform the digestions sequentially. Perform the first digestion reaction (including both enzymes) in 1X Tango buffer, then add an additional aliquot of 10X Tango buffer (1/8 of initial reaction volume) to reach a final 2X Tango buffer concentration and continue digestion with the second enzyme.
3. **Double Digestion using Color-Coded Buffers**
   - If possible, use the buffer in which both enzymes have 100% activity.
   - If this is not possible, choose the buffer in which both enzymes maintain at least 20% of their activity. Increase the amount of the enzymes in your digest according to their activity in that buffer.
   - For enzymes that are prone to relaxation of site specificity, use a buffer in which they do not exhibit star activity.

1X, 2X – Final buffer concentration.
(65°C) – Incubation temperature if other than 37°C.
Tail (MaeII)neo – Tail is a neoschizomer of MaeII; same recognition specificity, but different cleavage pathern.
100* – Star activity appears at a greater than 5-fold overdigestion (5 units x 1 hour).
NR – Buffer is not recommended because of high star activity.
★ – Enzyme is prone to star activity.
65 / 80 / No – Thermal inactivation conditions.
80 (≤10 u) – Up to 10 units of enzyme can be inactivated at 80°C in 20 min.
Dam Dcm CG – Cleavage blocked or impaired by Dam, Dcm or CpG methylation within the recognition sequence.
Dam Dcm CG – Overlapping Dam, Dcm or CpG methylation; may block or impair DNA cleavage.

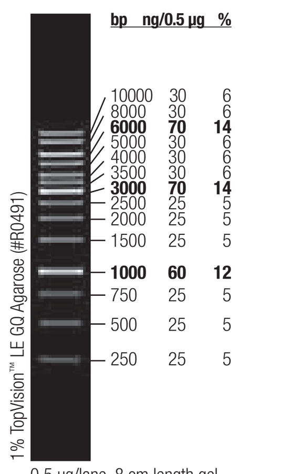
Single letter code – B = C, G or T; D = A, G or T; H = A, C or T; K = G or T; M = A or C; N = G, A, T or C; R = G or A; S = C or G; V = A, C or G; W = A or T; Y = C or T

Buffer composition

Buffer	Composition
1X Buffer B	10 mM Tris-HCl (pH 7.5 at 37°C), 10 mM $MgCl_2$, 0.1 mg/mL BSA
1X Buffer G	10 mM Tris-HCl (pH 7.5 at 37°C), 10 mM $MgCl_2$, 50 mM NaCl, 0.1 mg/mL BSA
1X Buffer O	50 mM Tris-HCl (pH 7.5 at 37°C), 10 mM $MgCl_2$, 100 mM NaCl, 0.1 mg/mL BSA
1X Buffer R	10 mM Tris-HCl (pH 8.5 at 37°C), 10 mM $MgCl_2$, 100 mM KCl, 0.1 mg/mL BSA
1X Buffer Tango	33 mM Tris-acetate (pH 7.9 at 37°C), 10 mM Mg-acetate, 66 mM K-acetate, 0.1 mg/mL BSA
2X Buffer Tango	66 mM Tris-acetate (pH 7.9 at 37°C), 20 mM Mg-acetate, 132 mM K-acetate, 0.2 mg/mL BSA
Unique Buffer	For composition of unique buffers please refer to thermoscientific.com/onebio

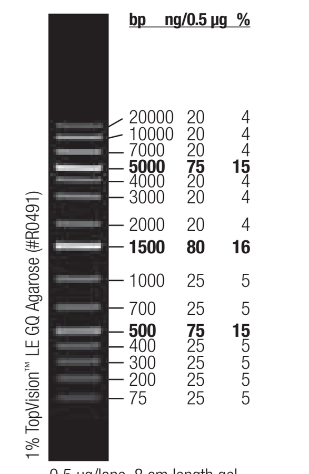
Thermo Scientific™ GeneRuler™ and O'GeneRuler™ DNA Ladders

GeneRuler™ 1 kb DNA Ladder #SM0311/2/3
O'GeneRuler™ 1 kb DNA Ladder, ready-to-use #SM1163



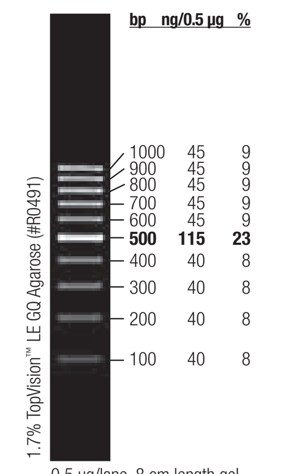
0.5 µg/lane, 8 cm length gel, 1X TAE, 7V/cm, 45 min

GeneRuler™ 1 kb Plus DNA Ladder #SM1331/2/3
O'GeneRuler™ 1 kb Plus DNA Ladder, ready-to-use #SM1343



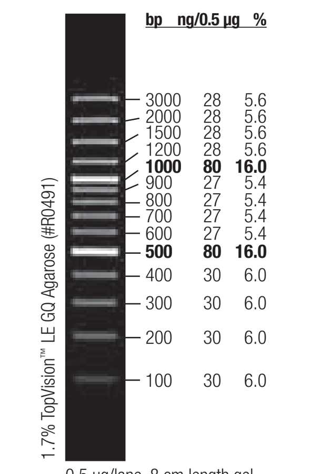
0.5 µg/lane, 8 cm length gel, 1X TAE, 7V/cm, 45 min

GeneRuler™ 100 bp DNA Ladder #SM0241/2/3
O'GeneRuler™ 100 bp DNA Ladder, ready-to-use #SM1143



0.5 µg/lane, 8 cm length gel, 1X TBE, 5V/cm, 1 h

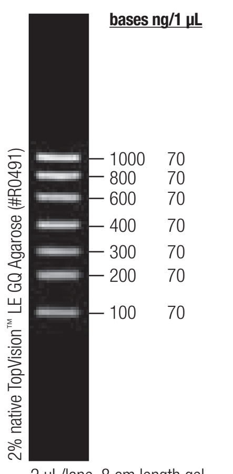
GeneRuler™ 100 bp Plus DNA Ladder #SM0321/2/3
O'GeneRuler™ 100 bp Plus DNA Ladder, ready-to-use #SM1153



0.5 µg/lane, 8 cm length gel, 1X TBE, 5V/cm, 1 h

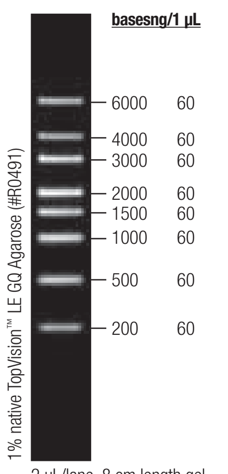
Thermo Scientific™ RiboRuler™ RNA Ladders

RiboRuler™ Low Range RNA Ladder #SM1831/3



2 µL/lane, 8 cm length gel, 1X TAE, 5V/cm

RiboRuler™ High Range RNA Ladder #SM1821/3



2 µL/lane, 8 cm length gel, 1X TAE, 5V/cm

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