Fermentas Restriction Endonucleases Activity

in the Five Buffer Plus System

Enzyme	Prototype	Specificity 5'→3'	B+ (blue) 1X	G+ (green) 1X	O+ (orange) 1X	R+ (red) 1X	Y+/Tango (yellow) 1X	Y+/Tango (yellow) 2X	Y+/Tango buffer concentration for double digestion	Units for overnight incubation and other enzyme properties
AarI	AarI	CACCTGC(N)4/8↓	NR(20-50)	NR(100)	0-20	0-20	NR(50-100)	50-100	2X	1.0
AasI	DrdI	GACNNNN↓NNGTC	100	20-50	0-20	0-20	50-100*	0-20	1X*	0.1
AatII	AatII	GACGT↓C	50-100	20-50	0-20	0-20	100	20-50	1X or 2X	0.3
Acc65I		G↓GTACC	0-20	20-50	100	20-50	20-50	50-100	1X or 2X	0.3
	(KpnI)	GGTAC↓C								
AdeI	DraIII	CACNNN↓GTG	0-20	100	20-50	100	100*	20-50	1X* or 2X	0.5
AloI (30°C)	AloI	↓7/12-13(N)GAAC(N)6TCC(N)12-13/7↓	0-20	0-20	0-20	100	20-50	100	1X or 2X	0.1
AluI	AluI	AG↓CT	50-100	0-20	0-20	0-20	100	20-50	1X or 2X	0.1
Alw21I	HgiAI	GWGCW↓C	0-20	20-50	100	50-100	20-50	50-100	1X or 2X	0.1
Alw26I	BsmAI	GTCTC(N)1/5↓	50-100	100	0-20	0-20	100	100	1X or 2X	0.2
Alw44I	ApaLI	G↓TGCAC	50-100	100	0-20	50-100	100	50-100	1X or 2X	0.1
ApaI	ApaI	GGGCC↓C	100	20-50	0-20	0-20	20-50	0-20	1X	0.2
BamHI	BamHI	G↓GATCC	20-50*	100	20-50	50-100*	100*	50-100	1X* or 2X	0.5
BclI (55°C)	BclI	T↓GATCA	20-50	100	20-50	20-50	100*	100	1X* or 2X	0.1
BcnI	CauII	CC↓SGG	20-50	50-100	50-100	50-100	100	50-100	1X or 2X	0.2
BcuI	SpeI	A↓CTAGT	50-100	50-100	0-20	20-50	100	0-20	1X	0.5
BfiI	BfiI	ACTGGG(N)5/4↓	20-50	20-50	0-20	0-20	100	0-20	1X	1.0
BfmI	SfeI	C↓TRYAG	0-20	50-100	0-20	0-20	100	20-50	1X or 2X	1.0
BfuI	BciVI	GTATCC(N)6/5↓	NR(50-100)	NR(50-100)	0-20	0-20	NR(100)	50-100*	2X*	1.0
BglI	BglI	GCCNNNN↓NGGC	0-20	50-100	100	100	0-20	100	2X	0.1
BglII	BglII	A↓GATCT	0-20	20-50	100	50-100	0-20	100	2X	0.1
Bme1390I	ScrFI	CC↓NGG	20-50	50-100	100	50-100	50-100	50-100	1X or 2X	0.2
BoxI	PshAI	GACNN↓NNGTC	0-20	0-20	0-20	20-50	100	20-50	1X or 2X	0.5
BpiI	BbvII	GAAGAC(N)2/6↓	20-50	100	50-100	50-100	50-100	50-100	1X or 2X	0.3
BplI	BplI	↓8/13(N)GAG(N)5CTC(N)13/8↓	0-20(+SAM)	20-50(+SAM)	0-20(+SAM)	0-20(+SAM)	100(+SAM)	20-50(+SAM)	1X(+SAM) or 2X(+SAM)	0.3
Bpu10I	Bpu10I	CCTNAGC-5/-2↓	0-20	20-50*	50-100*	100*	50-100*	100*	1X* or 2X*	0.2
Bpu1102I	EspI	GC↓TNAGC	50-100	50-100	20-50	20-50	100	20-50	1X or 2X	0.1
BseDI (55°C)	SecI	C↓CNNGG	50-100	20-50	0-20	0-20	100	50-100	1X or 2X	0.2
BseGI (55°C)		GGATG(N)2/0↓	20-50	50-100	20-50	20-50	100	20-50	1X or 2X	0.2
	(FokI)	GGATG(N)9/13↓								
BseJI (65°C)	BsaBI	GATNN↓NNATC	NR(20-50)	100*	100	NR(100)	NR(100)	100*	2X*	0.1
BseLI (55°C)	BsiYI	CCNNNNN↓NNGG	20-50	100	0-20	20-50	100	50-100	1X or 2X	0.1
BseMI (55°C)	BsrDI	GCAATG(N)2/0↓	0-20	20-50	0-20	100	50-100	50-100	1X or 2X	0.3
BseMII (55°C)	BseMII	CTCAG(N)10/8↓	50-100(+SAM)	50-100(+SAM)	50-100(+SAM)	50-100(+SAM)	100(+SAM)	50-100(+SAM)	1X(+SAM) or 2X(+SAM)	0.5
BseNI (65°C)	BsrI	ACTGG(N)1/-1↓	100	20-50	0-20	0-20	50-100	20-50	1X or 2X	0.1
BseSI (55°C)	BseSI	GKGCM↓C	20-50	100	0-20	20-50	50-100	0-20	1X	0.1
BseXI (65°C)	BbvI	GCAGC(N)8/12↓	0-20	NR(20-50)	NR(100)	NR(100)	NR(50-100)	NR(100)	not recommended	0.3
Bsh1236I	FnuDII	CG↓CG	0-20	0-20	50-100	100	20-50	50-100	1X or 2X	0.1
Bsh1285I	McrI	CGRY↓CG	20-50	100	20-50	0-20	0-20	20-50	2X	0.2
BshNI	HgiCI	G↓GYRCC	0-20	20-50	100	50-100	0-20	100	2X	0.3
BshTI	AgeI	A↓CCGGT	0-20	20-50	100	50-100	20-50	20-50	1X or 2X	0.1
Bsp68I	NruI	TCG↓CGA	0-20	20-50	100	50-100	20-50	50-100	1X or 2X	0.1
Bsp119I	AsuII	TT↓CGAA	20-50	0-20	0-20	20-50	100	100	1X or 2X	0.1
Bsp120I		G↓GGCCC	100	20-50	0-20	20-50	50-100	0-20	1X	0.1
	(ApaI)	GGGCC↓C								
Bsp143I	MboI	↓GATC	20-50	20-50	0-20	0-20	50-100	20-50	1X or 2X	0.1
Bsp143II	HaeII	RGCGC↓Y	50-100*	20-50	0-20	20-50	100*	20-50	1X* or 2X	0.5
Bsp1407I	Bsp1407I	T↓GTACA	0-20	20-50	0-20	20-50	100	50-100	1X or 2X	0.1
BspLI	NlaIV	GGN↓NCC	50-100	50-100	0-20	20-50	100	20-50	1X or 2X	0.3
BspPI (55°C)	BinI	GGATC(N)4/5↓	20-50	20-50	0-20	0-20	100	0-20	1X	0.5
BspTI	AflII	C↓TTAAG	0-20	0-20	100	20-50	0-20	50-100	2X	0.1
Bst1107I	SnaI	GTA↓TAC	20-50	50-100	100	100	20-50	100	1X or 2X	0.1
BstXI (55°C)	BstXI	CCANNNNN↓NTGG	20-50	100	100	50-100	50-100	100	1X or 2X	0.1
Bsu15I	ClaI	AT↓CGAT	50-100	20-50	0-20	20-50	100	20-50	1X or 2X	0.1
BsuRI	HaeIII	GG↓CC	0-20	0-20	0-20	100	50-100	100	1X or 2X	0.1
CaiI	AlwNI	CAGNNN↓CTG	20-50	20-50	20-50	50-100	100	50-100	1X or 2X	0.2
CfrI	CfrI	Y↓GGCCR	50-100*	50-100	0-20	0-20	100	0-20	1X	1.0
Cfr9I		C↓CCGGG	0-20	0-20	0-20	0-20	20-50	0-20	1X	0.2
	(SmaI)	CCC↓GGG								
Cfr10I	Cfr10I	R↓CCGGY	0-20	20-50	20-50	50-100	20-50	50-100	1X or 2X	0.1
Cfr13I	AsuI	G↓GNCC	50-100	50-100	20-50	20-50	100	20-50	1X or 2X	0.3
Cfr42I	SacII	CCGC↓GG	100	50-100	0-20	0-20	50-100	0-20	1X	0.1
CpoI	RsrII	CG↓GWCCG	20-50	50-100	50-100	20-50	100	50-100	1X or 2X	0.5
Csp6I		G↓TAC	100	50-100	0-20	0-20	50-100	0-20	1X	0.1
	(RsaI)	GT↓AC								
DpnI	DpnI	Gm6A↓TC	100	100	50-100	50-100	100	50-100	1X or 2X	0.1
DraI	AhaIII	TTT↓AAA	100	50-100	0-20	20-50	50-100	50-100	1X or 2X	0.1
Eam1104I	Ksp632I	CTCTTC(N)1/4↓	50-100	50-100	0-20	0-20	100	0-20	1X	0.5
Eam1105I	Eam1105I	GACNNN↓NNGTC	20-50	50-100	0-20	0-20	50-100	20-50	1X or 2X	0.1
Ecl136II		GAG↓CTC	50-100	20-50	0-20	0-20	50-100	0-20	1X	0.2
	(SacI)	GAGCT↓C								
Eco24I	HgiJII	GRGCY↓C	50-100	50-100	20-50	20-50	100	0-20	1X	0.2
Eco31I	Eco31I	GGTCTC(N)1/5↓	50-100	100	0-20	0-20	50-100	20-50	1X or 2X	0.3
Eco32I	EcoRV	GAT↓ATC	0-20	50-100	50-100	100	20-50	100	1X or 2X	0.1
Eco47I	AvaII	G↓GWCC	0-20	50-100	50-100	100	50-100	50-100	1X or 2X	0.3
Eco47III	Eco47III	AGC↓GCT	0-20	20-50	100	100	50-100	100	1X or 2X	0.1
Eco52I	XmaIII	C↓GGCCG	0-20	0-20	0-20	20-50	0-20	20-50	2X	0.2
Eco57I	Eco57I	CTGAAG(N)16/14↓	100(+SAM)	100(+SAM)	20-50(+SAM)	20-50(+SAM)	50-100(+SAM)	50-100(+SAM)	1X(+SAM) or 2X(+SAM)	1.0
Eco57MI	Eco57MI	CTGRAG(N)16/14↓	100(+SAM)	50-100(+SAM)	0-20(+SAM)	20-50(+SAM)	50-100(+SAM)	0-20(+SAM)	1X(+SAM)	1.0
Eco72I	PmaCI	CAC↓GTG	50-100	100	0-20	0-20	100	20-50	1X or 2X	0.5
Eco81I	SauI	CC↓TNAGG	50-100	100	0-20	0-20	100	0-20	1X	0.1
Eco88I	AvaI	C↓YCGRG	100	50-100	0-20	0-20	100	20-50	1X or 2X	0.2
Eco91I	BstEII	G↓GTNACC	20-50	20-50	100	50-100	50-100	100	1X or 2X	0.1
Eco105I	SnaBI	TAC↓GTA	100*	50-100	0-20	0-20	100	0-20	1X	0.5
Eco130I	StyI	C↓CWWGG	0-20	20-50	100	50-100	50-100	100	1X or 2X	0.2
Eco147I	StuI	AGG↓CCT	100	50-100	20-50	20-50	50-100	0-20	1X	0.1
Eco0109I	DraII	RG↓GNCCY	50-100	20-50	20-50	20-50	100	100	1X or 2X	0.2

Enzyme	Prototype	Specificity 5'→3'	B+ (blue) 1X	G+ (green) 1X	O+ (orange) 1X	R+ (red) 1X	Y+/Tango (yellow) 1X	Y+/Tango (yellow) 2X	Y+/Tango buffer concentration for double digestion	Units for overnight incubation and other enzyme properties
EcoRI	EcoRI	G↓AATTC	0-20	NR(20-50)	100	100*	NR(100)	100	2X	0.2
EheI		GGC↓GCC	20-50	50-100	0-20	0-20	100	20-50	1X or 2X	1.0
	(NarI)	GG↓CGCC								
Esp3I	Esp3I	CGTCTC(N)1/5↓	100(+DTT)	20-50(+DTT)	0-20(+DTT)	0-20(+DTT)	100(+DTT)	0-20(+DTT)	1X(+DTT)	0.2
FspAI	FspAI	RTGC↓GCAY	0-20	0-20	100	50-100	0-20	50-100	2X	0.2
GsuI (30°C)	GsuI	CTGGAG(N)16/14↓	100	50-100	0-20	20-50	100	50-100	1X or 2X	1.0
Hin1I	AcyI	GR↓CGYC	20-50	100	20-50	20-50	20-50	20-50	1X or 2X	0.1
Hin4I	Hin4I	↓8/13-14(N)GAY(N)5VTC(N)13-14/8↓	20-50(+SAM)	20-50(+SAM)	0-20(+SAM)	0-20(+SAM)	100(+SAM)	0-20(+SAM)	1X(+SAM)	0.2
Hin6I		G↓CGC	50-100	50-100	20-50	20-50	100	50-100	1X or 2X	0.1
	(HhaI)	GCG↓C								
HincII	HindII	GTY↓RAC	50-100	50-100	20-50	50-100	100	50-100	1X or 2X	0.1
HindIII	HindIII	A↓AGCTT	0-20	20-50	0-20	100	50-100	50-100	1X or 2X	0.1
HinfI	HinfI	G↓ANTC	0-20	20-50	50-100	100	50-100	50-100	1X or 2X	0.1
HpaII	HpaII	C↓CGG	50-100	50-100	0-20	20-50	100	20-50	1X or 2X	0.1
HphI	HphI	GGTGA(N)8/7↓	100	0-20	0-20	0-20	20-50	0-20	1X	0.1
Hpy8I	MjaIV	GTN↓NAC	50-100	50-100	0-20	20-50	100	50-100	1X or 2X	0.1
HpyF10VI	MwoI	GCNNNNN↓NNGC	0-20	0-20	0-20	0-20	100	50-100	1X or 2X	0.1
KpnI	KpnI	GGTAC↓C	20-50	0-20	0-20	0-20	20-50	0-20	1X	0.2
Kpn2I (55°C)	BspMII	T↓CCGGA	50-100	50-100	0-20	20-50	100	50-100	1X or 2X	0.1
KspAI	HpaI	GTT↓AAC	100	50-100*	20-50	20-50	100*	50-100	1X* or 2X	0.5
LweI	SfaNI	GCATC(N)5/9↓	0-20	0-20	0-20	20-50	100	20-50	1X or 2X	0.2
MbiI	BsrBI	GAGCGG-3/-3↓	20-50	100	0-20	20-50	100	20-50	1X or 2X	0.2
MboI	MboI	↓GATC	50-100	50-100	50-100	100	50-100	100	1X or 2X	0.1
MboII	MboII	GAAGA(N)8/7↓	100	50-100	20-50	0-20	50-100	20-50	1X or 2X	1.0
MlsI	BalI	TGG↓CCA	0-20	20-50	0-20	100	20-50	50-100	1X or 2X	0.5
MluI	MluI	A↓CGCGT	0-20	20-50	50-100	100	20-50	50-100	1X or 2X	0.1
MnlI	MnlI	CCTC(N)7/6↓	50-100	100	20-50	20-50	20-50	20-50	1X or 2X	0.5
Mph1103I	AvaIII	ATGCA↓T	0-20	50-100	20-50	100	50-100	50-100	1X or 2X	0.3
MspI	HpaII	C↓CGG	50-100	50-100	0-20	0-20	100	50-100	1X or 2X	0.3
MssI	PmeI	GTTT↓AAAC	100	0-20	0-20	0-20	20-50	0-20	1X	0.5
MunI	MfeI	C↓AATTG	100	100	0-20	0-20	100	0-20	1X	0.1
MvaI		CC↓WGG	20-50	20-50	50-100	100	20-50*	100	1X* or 2X	0.1
	(EcoRII)	↓CCWGG								
Mva1269I	BsmI	GAATGC(N)1/-1↓	0-20	20-50	50-100	100	0-20	50-100	2X	0.1
NcoI	NcoI	C↓CATGG	20-50	20-50	20-50	50-100	100	100	1X or 2X	0.1
NdeI	NdeI	CA↓TATG	0-20	0-20	50-100	100	0-20	50-100	2X	0.3
NheI	NheI	G↓CTAGC	100	20-50	0-20	0-20	100	0-20	1X	0.2
NmuCI	Tsp45I	↓GTSAC	0-20	20-50	50-100	100	20-50	50-100	1X or 2X	0.1
NotI	NotI	GC↓GGCCGC	0-20	0-20	100	20-50	0-20	20-50	2X	0.1
NsbI	MstI	TGC↓GCA	20-50	50-100	0-20	20-50	100	20-50	1X or 2X	0.1
OliI	OliI	CACNN↓NNGTG	0-20	0-20	0-20	100	0-20	50-100	2X	0.2
PaeI	SphI	GCATG↓C	100	50-100	0-20	0-20	20-50	0-20	1X	0.1
PagI	BspHI	T↓CATGA	0-20	50-100	100	NR(20-50)	NR(20-50)	NR(20-50)	not recommended	0.2
PauI	BsePI	G↓CGCGC	0-20	0-20	100	100	0-20	100	2X	0.2
PdiI	NaeI	GCC↓GGC	50-100	20-50	0-20	0-20	100	50-100	1X or 2X	0.5
PdmI	XmnI	GAANN↓NNTTC	20-50	50-100	0-20	0-20	100	0-20	1X	0.5
Pfl23II	SplI	C↓GTACG	20-50	50-100	20-50	20-50	100	0-20	1X	0.3
PpiI (30°C)	PpiI	↓7/12(N)GAAC(N)5CTC(N)13/8↓	0-20	0-20	0-20	100	50-100	50-100	1X or 2X	0.1
Psp5II	PpuMI	RG↓GWCCY	0-20	100	20-50	20-50	50-100	100	1X or 2X	0.2
Psp1406I	AclI	AA↓CGTT	100	50-100	0-20	20-50	100	0-20	1X	0.5
PstI	PstI	CTGCA↓G	50-100	50-100	100	100	50-100	50-100	1X or 2X	0.2
PsuI	XhoII	R↓GATCY	100	20-50	0-20	0-20	50-100	0-20	1X	0.5
PsyI	Tth111I	GACN↓NNGTC	100	50-100	0-20	0-20	50-100	0-20	1X	0.1
PvuI	PvuI	CGAT↓CG	0-20	20-50	50-100	100	50-100	100	1X or 2X	0.2
PvuII	PvuII	CAG↓CTG	50-100*	100	20-50	50-100	20-50*	20-50*	1X* or 2X*	0.2
RsaI	RsaI	GT↓AC	50-100	20-50	0-20	0-20	100	0-20	1X	0.2
SacI	SacI	GAGCT↓C	50-100	20-50	0-20	0-20	50-100	20-50	1X or 2X	0.2
SalI	SalI	G↓TCGAC	0-20	0-20	100	20-50	0-20	50-100	2X	0.1
SatI	Fnu4HI	GC↓NGC	20-50	100	20-50	20-50	50-100	20-50	1X or 2X	0.1
ScaI	ScaI	AGT↓ACT	0-20	0-20	0-20	NR(50-100)	0-20	NR(20-50)	not recommended	0.3
SchI		GAGTC(N)5/5↓	20-50	50-100	0-20	0-20	100	0-20	1X	0.2
	(PleI)	GAGTC(N)4/5↓								
SdaI	Sse8387I	CCTGCA↓GG	NR(100)	100*	0-20	0-20	NR(100)	20-50	2X	0.3
SduI	SduI	GDGCH↓C	NR(50-100)	50-100*	0-20	0-20	NR(100)	NR(50-100)	not recommended	0.3
SmaI (30°C)	SmaI	CCC↓GGG	50-100	0-20	0-20	0-20	100	0-20	1X	0.2
SmiI (30°C)	SwaI	ATTT↓AAAT	0-20	0-20	100	20-50	0-20	20-50	2X	0.1
SmuI	FauI	CCCGC(N)4/6↓	50-100	50-100	0-20	20-50	100	20-50	1X or 2X	0.2
SspI	SspI	AAT↓ATT	20-50	100	0-20	50-100	100	20-50	1X or 2X	0.1
TaaI (65°C)	Tsp4CI	ACN↓GT	0-20	0-20	0-20	50-100	100	100	1X or 2X	0.2
TaiI (65°C)		ACGT↓	50-100	50-100	20-50	100	100	50-100	1X or 2X	0.3
	(MaeII)	A↓CGT								
TaqI (65°C)	TaqI	T↓CGA	0-20	20-50	20-50	20-50	20-50	20-50	1X or 2X	0.3
TasI (65°C)	TspEI	↓AATT	100	50-100	20-50	0-20	20-50	0-20	1X	0.3
TauI (55°C)	TauI	GCSG↓C	100	50-100	0-20	0-20	20-50	0-20	1X	1.0
TatI (65°C)	TatI	W↓GTACW	NR(50-100)	50-100*	0-20	20-50	100*	0-20	1X*	0.2
Tru1I (65°C)	MseI	T↓TAA	50-100	50-100	20-50	100	50-100	100	1X or 2X	0.2
Van91I	PflMI	CCANNNN↓NTGG	0-20	50-100	50-100	100	20-50	50-100	1X or 2X	0.1
VspI	VspI	AT↓TAAT	0-20	50-100	100	20-50	100	100	1X or 2X	0.1
XagI	EcoNI	CCTNN↓NNNAGG	0-20	20-50	50-100	100	20-50	50-100	1X or 2X	0.1
XapI	ApoI	R↓AATTY	50-100	100	0-20	0-20	100	20-50	1X or 2X	0.1
XbaI	XbaI	T↓CTAGA	50-100	50-100	20-50	0-20	100	50-100	1X or 2X	0.1
XceI	NspI	RCATG↓Y	50-100	0-20	0-20	0-20	100	0-20	1X	0.2
XhoI	XhoI	C↓TCGAG	0-20	50-100	50-100	100	20-50	100	1X or 2X	0.1
XmaJI	AvrII	C↓CTAGG	20-50	50-100	50-100	50-100	100	50-100	1X or 2X	0.2
XmiI	AccI	GT↓MKAC	100	0-20	0-20	0-20	50-100	20-50	1X or 2X	0.1
N.Bpu10I	N.Bpu10I	GC↓TNAGG CG ANTCC	0-20	20-50	20-50	100	20-50	50-100	1X or 2X	0.3

1X Buffer B+	10mM Tris-HCl (pH 7.5 at 37°C), 10mM $MgCl_2$ and 0.1mg/ml BSA.
1X Buffer G+	10mM Tris-HCl (pH 7.5 at 37°C), 10mM $MgCl_2$, 50mM NaCl and 0.1mg/ml BSA.
1X Buffer O+	50mM Tris-HCl (pH 7.5 at 37°C), 10mM $MgCl_2$, 100mM NaCl and 0.1mg/ml BSA.
1X Buffer R+	10mM Tris-HCl (pH 8.5 at 37°C), 10mM $MgCl_2$, 100mM KCl and 0.1mg/ml BSA.
Buffer Y+/Tango	
1X	33mM Tris-acetate (pH 7.9 at 37°C), 10mM Mg-acetate, 66mM K-acetate and 0.1mg/ml BSA.
2X	66mM Tris-acetate (pH 7.9 at 37°C), 20mM Mg-acetate, 132mM K-acetate and 0.2mg/ml BSA.
For unique buffer composition *see* 2002/2003 Catalog pp.100-101.	

1X, 2X Indicates final buffer concentration.
(30°C) Incubate as indicated, if not – incubate at 37°C.
(*ApaI*) Indicates an enzyme with cleavage specificity different from prototype.
* Star activity appears at 10-fold overdigestion (10 units x 1 hour).
NR Buffer is not recommended, since star activity appears at 5-fold overdigestion (5 units x 1 hour).
Restriction enzyme exhibits star activity under certain conditions.
Denotes the possibility of thermal inactivation (at 65°C or 80°C in 20min).
Indicates that only small amounts of enzyme (up to 10 units) can be inactivated at 80°C in 20min.
Cleavage blocked by Dam or Dcm methylation of target DNA.
Overlapping Dam or Dcm methylation may influence DNA cleavage.
Cleavage blocked by CG methylation.
Overlapping CG methylation may influence DNA cleavage.

How do I do a Double Digest?

1. Select each enzyme of interest.
2. If the optimal color-coded buffer for both enzymes is the same, use that buffer.
3. If not, use the buffer in which both enzymes are most active.
4. If neither buffer is suitable for both REs, refer to the column headed "**Y+/Tango buffer concentration for double digestion**".
   - If the same Y+/Tango buffer concentration is suitable for both enzymes, use it. If both 1X and 2X are suitable, choose 2X.
   - If different Y+/Tango buffer concentrations are recommended, perform the first digest at 1X final concentration, then add extra 10X Y+/Tango buffer (0.125 x initial reaction volume) to bring the final buffer concentration to 2X to perform the second digest.
   - If Y+/Tango buffer is "not recommended" (star activity appears at a 5-fold overdigestion (5u x 1 hour)), perform sequential digests in REs optimal buffers.
   - "*" indicates the appearance of star activity at a 10-fold overdigestion (10 units x 1 hour). To avoid this, use a smaller excess of RE.

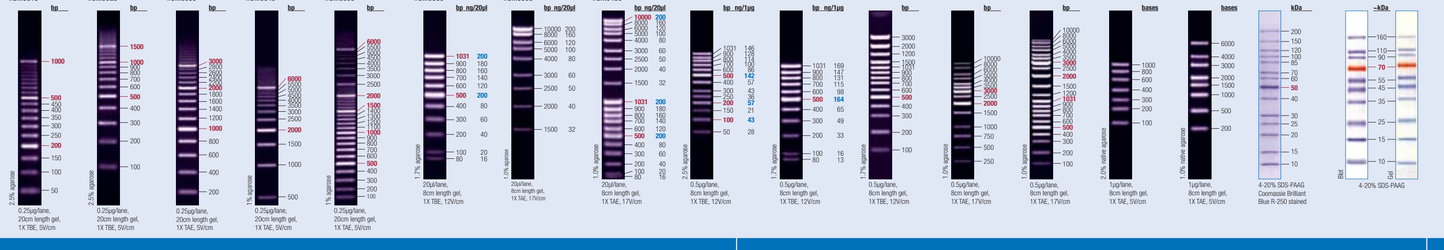
Activity of DNA/RNA Modifying Enzymes in the Five Buffer Plus System

Enzyme	B+ (blue) 1X	G+ (green) 1X	O+ (orange) 1X	R+ (red) 1X	Y+/Tango (yellow) 1X	Y+/Tango (yellow) 2X	BamHI+	Ecl136II+ SacI+	KpnI+
DNA Polymerase I, *E.coli*	25-50	75-100	100	100	100	100	100	50-75	50-75
Klenow Fragment	25-50	25-50	100	100	100	100	100	50-75	50-75
Klenow Fragment, exo⁻	25-50	25-50	100	100	100	100	100	50-75	50-75
T4 DNA Polymerase	75-100	75-100	100	100	100	100	100	100	100
T7 DNA Polymerase	75-100	100	100	100	100	100	75-100	75-100	100
T4 DNA Ligase*	100	100	75-100	75-100	75-100	75-100	75-100	50	100
T4 Polynucleotide Kinase	75-100	100	100	75-100	100	100	100	50-75	75-100
T4 Polynucleotide Kinase, 3'-phosphatase Minus	100	100	100	75-100	100	100	100	75-100	100
Exonuclease III	100	25-50	0-25	0-25	25-50	0-25	0-25	100	100
Bacterial Alkaline Phosphatase	75-100	100	100	100	100	100	100	50-75	75-100
Calf Intestine Alkaline Phosphatase	100	100	100	100	100	100	100	25-50	100
Shrimp Alkaline Phosphatase	100	100	100	100	100	100	100	75-100	100

* Buffers were supplemented with 0.5mM ATP, since T4 DNA Ligase is an ATP-dependent enzyme.

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

DNA, RNA & Protein Ladders



- O'RangeRuler™ 50bp DNA Ladder, ready-to-use #SM0613 — 2.5% agarose; 0.25µg/lane, 20cm length gel, 1X TBE, 5V/cm
- O'RangeRuler™ 100bp DNA Ladder, ready-to-use #SM0623 — 2.5% agarose; 0.25µg/lane, 20cm length gel, 1X TBE, 5V/cm
- O'RangeRuler™ 200bp DNA Ladder, ready-to-use #SM0633 — 0.25µg/lane, 20cm length gel, 1X TAE, 5V/cm
- O'RangeRuler™ 500bp DNA Ladder, ready-to-use #SM0643 — 1% agarose; 0.25µg/lane, 20cm length gel, 1X TAE, 5V/cm
- O'RangeRuler™ 100+500bp DNA Ladder, ready-to-use #SM0653 — 1% agarose; 0.25µg/lane, 20cm length gel, 1X TAE, 5V/cm
- MassRuler™ DNA Ladder, Low Range, ready-to-use #SM0383 — 1.7% agarose; 20µl/lane, 8cm length gel, 1X TBE, 12V/cm
- MassRuler™ DNA Ladder, High Range, ready-to-use #SM0393 — 1.0% agarose; 20µl/lane, 8cm length gel, 1X TAE, 17V/cm
- MassRuler™ DNA Ladder, Mix, ready-to-use #SM0403 — 1.0% agarose; 20µl/lane, 8cm length gel, 1X TAE, 17V/cm
- GeneRuler™ 50bp DNA Ladder #SM0371/2/3 — 2.5% agarose; 0.5µg/lane, 8cm length gel, 1X TBE, 12V/cm
- GeneRuler™ 100bp DNA Ladder #SM0241/2/3 — 1.7% agarose; 0.5µg/lane, 8cm length gel, 1X TBE, 12V/cm
- GeneRuler™ 100bp DNA Ladder Plus #SM0321/2/3 — 1.7% agarose; 0.5µg/lane, 8cm length gel, 1X TBE, 12V/cm
- GeneRuler™ 1kb DNA Ladder #SM0311/2/3 — 1.0% agarose; 0.5µg/lane, 8cm length gel, 1X TAE, 17V/cm
- GeneRuler™ DNA Ladder Mix #SM0331/2/3 — 1.0% agarose; 0.5µg/lane, 8cm length gel, 1X TAE, 17V/cm
- RNA Ladder, Low Range #SM0411/3 — 2.0% native agarose; 1µg/lane, 8cm length gel, 1X TAE, 5V/cm
- RNA Ladder, High Range #SM0421/3 — 1.0% native agarose; 1µg/lane, 8cm length gel, 1X TAE, 5V/cm
- Protein Ladder, 10-200kDa #SM0661 — 4-20% SDS-PAAG Coomassie Brilliant Blue R-250 stained
- Prestained Protein Ladder, ~10-160kDa #SM0671 — Blot / Gel; 4-20% SDS-PAAG

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