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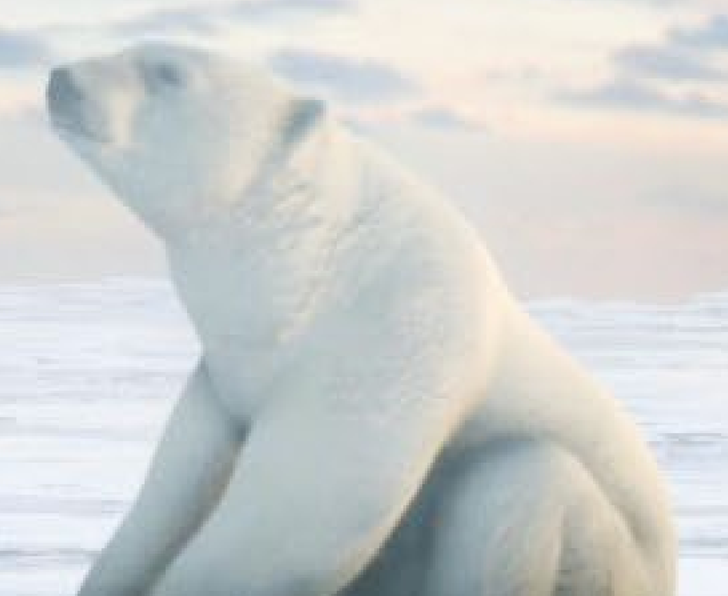

Performance Chart for Restriction Enzymes

Icon codes used in the table below: R = Recombinant; TS = Time-Saver qualified; e = Engineered for maximum performance; 2+site = requires two or more sites for cleavage (see Chart Legend).

Icons	Enzyme	Supplied NEBuffer	% Activity in NEBuffers r1.1	r2.1	r3.1	rCutSmart	Incub. Temp. (°C)	Inactiv. Temp. (°C)	Dil.	Unit Substrate	Methylation Sensitivity	Notes
R, TS	AatII	rCutSmart	<10	50*	50	100	37°	80°	B	λ DNA	CpG	
R	AbaSI	rCutSmart + DTT	25	50	50	100	25°	65°	C	T4 wild-type phage DNA (fully ghmC-modified)		e
R, TS	Acc65I	r3.1	10	75*	100	25	37°	65°	A	pBC4 DNA	dcm, CpG	
R, TS	AccI	rCutSmart	50	50	10	100	37°	80°	A	λ DNA	CpG	
R, TS	AciI	rCutSmart	<10	25	100	100	37°	65°	A	λ DNA	CpG	
R, TS	AclI	rCutSmart	<10	<10	<10	100	37°	No	B	λ DNA	CpG	
R, TS	AcuI	rCutSmart	50	100	50	100	37°	65°	B	λ DNA		1, b, d
R	AfeI	rCutSmart	25	100	25	100	37°	65°	B	pXba DNA	CpG	
R, TS	AflII	rCutSmart	50	100	10	100	37°	65°	A	ΦX174 RF I DNA		
R, TS	AflIII	r3.1	10	50	100	50	37°	80°	B	λ DNA		
R, TS, e	AgeI-HF	rCutSmart	100	50	10	100	37°	65°	A	λ DNA	CpG	
R, TS	AhdI	rCutSmart	25	25	10	100	37°	65°	A	λ DNA	CpG	a
R, e	AleI-v2	rCutSmart	<10	<10	<10	100	37°	65°	B	λ DNA	CpG	
R, TS	AluI	rCutSmart	25	100	50	100	37°	80°	B	λ DNA		b
R	AlwI	rCutSmart	50	50	10	100	37°	No	A	λ DNA (dam-)	dam	1, b, d
R, TS	AlwNI	rCutSmart	10	100	50	100	37°	80°	A	λ DNA	dcm	
R, TS	ApaI	rCutSmart	25	25	<10	100	37°	65°	A	pXba DNA	dcm, CpG	
R, TS	ApaLI	rCutSmart	100	100	10	100	37°	No	A	λ DNA (HindIII digest)	CpG	
R, TS	ApeKI	r3.1	25	50	100	10	75°	No	B	λ DNA	CpG	
R, TS, e	ApoI-HF	rCutSmart	10	100	10	100	37°	80°	B	λ DNA		
R, TS	AscI	rCutSmart	<10	10	10	100	37°	80°	A	λ DNA	CpG	
R, TS	AseI	r3.1	<10	50*	100	10	37°	65°	B	λ DNA		3
R	AsiSI	rCutSmart	100	100	25	100	37°	80°	B	XhoI digested pXba	CpG	2, b
R, TS	AvaI	rCutSmart	<10	100	25	100	37°	80°	A	λ DNA	CpG	
R, TS	AvaII	rCutSmart	50	75	10	100	37°	80°	A	λ DNA	dcm, CpG	
R, TS	AvrII	rCutSmart	100	50	50	100	37°	No	B	λ DNA (HindIII digest)		
R, TS	BaeGI	r3.1	75	75	100	25	37°	80°	A	λ DNA		
R, TS	BaeI	rCutSmart	50	100	50	100	37°	65°	A	λ DNA	CpG	e
R, TS	BamHI	r3.1	75*	100*	100	100*	37°	No	A	λ DNA		3
R, TS, e	BamHI-HF	rCutSmart	100	50	10	100	37°	No	A	λ DNA		
R	BanI	rCutSmart	10	25	<10	100	37°	65°	A	λ DNA	dcm, CpG	1
R	BanII	rCutSmart	100	100	50	100	37°	80°	A	λ DNA		2
R, TS	BbsI	r2.1	100	100	25	75	37°	65°	B	λ DNA		
R, TS, e	BbsI-HF	rCutSmart	10	10	10	100	37°	65°	B	λ DNA		
R	BbvCI	rCutSmart	10	100	50	100	37°	No	B	λ DNA	CpG	1, a
R, TS, 2+site	BbvI	rCutSmart	100	100	25	100	37°	65°	B	pBR322 DNA		3
R	BccI	rCutSmart	100	50	10	100	37°	65°	A	pXba DNA		3, b
R	BceAI	r3.1	100*	100*	100	100*	37°	65°	A	pBR322 DNA	CpG	1
R, 2+site	BcgI	r3.1	10	75*	100	50*	37°	65°	A	λ DNA	dam, CpG	e
R, TS	BciVI	rCutSmart	100	25	<10	100	37°	80°	C	λ DNA		b
R, TS	BclI	r3.1	50	100	100	75	50°	No	A	λ DNA (dam-)	dam	
R, TS, e	BclI-HF	rCutSmart	100	100	10	100	37°	65°	B	λ DNA (dam-)	dam	
R, TS	BcoDI	rCutSmart	50	75	75	100	37°	No	B	λ DNA	CpG	
R	BfaI	rCutSmart	<10	10	<10	100	37°	80°	B	λ DNA		2, b
R, TS, 2+site	BfuAI	r3.1	<10	25	100	10	50°	65°	B	λ DNA	CpG	3
R, TS	BglI	r3.1	10	25	100	10	37°	65°	B	λ DNA	CpG	
R, TS	BglII	r3.1	10	10	100	<10	37°	No	A	λ DNA		
R, TS	BlpI	rCutSmart	50	100	10	100	37°	No	A	λ DNA		d
R, TS	BmgBI	r3.1	<10	10	100	10	37°	65°	B	λ DNA	CpG	3, b, d
R	BmrI	r2.1	75	100	75	100*	37°	65°	B	λ DNA (HindIII digest)		b
R, TS, e	BmtI-HF	rCutSmart	50	100	10	100	37°	65°	B	pXba DNA		
R, 2+site	BpmI	r3.1	75	100	100	100*	37°	65°	B	λ DNA		2
R	Bpu10I	r3.1	10	25	100	25	37°	80°	B	λ DNA		3, b, d
R, TS	BpuEI	rCutSmart	50*	100	50*	100	37°	65°	B	λ DNA		d
R, TS	BsaAI	rCutSmart	100	100	100	100	37°	No	C	λ DNA	CpG	
R	BsaBI	rCutSmart	50	100	75	100	60°	80°	B	λ DNA (dam-)	dam, CpG	2
R, TS	BsaHI	rCutSmart	50	100	100	100	37°	80°	C	λ DNA	dcm, CpG	
R, TS, e	BsaI-HFv2	rCutSmart	100	100	100	100	37°	80°	B	pXba DNA	dcm, CpG	
R	BsaJI	rCutSmart	50	100	100	100	60°	80°	A	λ DNA		
R, TS	BsaWI	rCutSmart	10	100	50	100	60°	80°	A	λ DNA		
TS	BsaXI	rCutSmart	50*	100*	10	100	37°	No	C	λ DNA		e
R, TS	BseRI	rCutSmart	100	100	75	100	37°	80°	A	λ DNA		d
R	BseYI	r3.1	10	50	100	50	37°	80°	B	λ DNA	CpG	d
R, TS, 2+site	BsgI	rCutSmart	25	50	25	100	37°	65°	B	λ DNA		d
R, TS	BsiEI	rCutSmart	25	50	<10	100	60°	No	A	λ DNA	CpG	
R, TS	BsiHKAI	rCutSmart	25	100	100	100	65°	No	A	λ DNA		
R, TS	BsiWI	r3.1	25	50*	100	25	55°	65°	B	ΦX174 DNA	CpG	
R, TS, e	BsiWI-HF	rCutSmart	50	100	10	100	37°	No	B	ΦX174 DNA	CpG	
R, TS	BslI	rCutSmart	50	75	100	100	55°	No	A	λ DNA	dcm, CpG	b
R, TS	BsmAI	rCutSmart	50	100	100	100	55°	No	B	λ DNA	CpG	
R, TS, e	BsmBI-v2	r3.1	<10	50	100	25	55°	80°	B	λ DNA	CpG	
R	BsmFI	rCutSmart	25	50	50	100	65°	80°	A	pBR322 DNA	dcm, CpG	1
R, TS	BsmI	rCutSmart	25	100	<10	100	65°	80°	A	λ DNA		
R, TS	BsoBI	rCutSmart	25	100	100	100	37°	80°	A	λ DNA		
R, TS	Bsp1286I	rCutSmart	25	25	25	100	37°	65°	A	λ DNA		3
R, TS	BspCNI	rCutSmart	100	75	10	100	37°	80°	A	λ DNA		b
R	BspDI	rCutSmart	25	75	50	100	37°	80°	A	λ DNA	dam, CpG	
R, TS	BspEI	r3.1	<10	10	100	<10	37°	80°	B	λ DNA (dam-)	dam, CpG	
R, TS	BspHI	rCutSmart	10	50	25	100	37°	80°	A	λ DNA	dam	
R, 2+site	BspMI	r3.1	10	50*	100	10	37°	65°	B	λ DNA		
R, TS	BspQI	r3.1	100*	100*	100	100*	50°	80°	B	λ DNA		3
R, TS	BsrBI	rCutSmart	50	100	100	100	37°	80°	A	λ DNA	CpG	d
R, TS	BsrDI	r2.1	10	100	75	25	37°	80°	A	λ DNA		3, d
R, TS, e	BsrFI-v2	rCutSmart	25	25	0	100	37°	No	C	pBR322 DNA	CpG	
R, TS, e	BsrGI-HF	rCutSmart	10	100	100	100	37°	80°	A	λ DNA		
TS	BsrI	r3.1	<10	50	100	10	65°	80°	B	ΦX174 DNA		b
R, TS	BssHII	rCutSmart	100	100	100	100	50°	65°	B	λ DNA	CpG	
R, TS, e	BssSI-v2	rCutSmart	10	25	<10	100	37°	No	B	λ DNA		
R	BstAPI	rCutSmart	50	100	25	100	60°	80°	A	λ DNA	CpG	b
R, TS	BstBI	rCutSmart	75	100	10	100	65°	No	A	λ DNA	CpG	
R, TS, e	BstEII-HF	rCutSmart	<10	10	<10	100	37°	No	A	λ DNA		
R, TS	BstNI	r3.1	10	100	100	75	60°	No	A	λ DNA		a
R, TS	BstUI	rCutSmart	50	100	25	100	60°	No	A	λ DNA	CpG	b
R, TS	BstXI	r3.1	<10	50	100	25	37°	80°	B	λ DNA	dcm	3
R, TS	BstYI	rCutSmart	25	100	75	100	60°	No	A	λ DNA		
R, TS, e	BstZ17I-HF	rCutSmart	100	100	10	100	37°	No	A	λ DNA	CpG	
R, TS	Bsu36I	rCutSmart	25	100	100	100	37°	80°	C	λ DNA (HindIII digest)		b
R, TS	BtgI	rCutSmart	50	100	100	100	37°	80°	B	pBR322 DNA		
R	BtgZI	rCutSmart	10	25	<10	100	60°	80°	A	λ DNA	CpG	3, b, d
R, TS	BtsCI	rCutSmart	10	100	25	100	50°	80°	B	λ DNA		
R, TS, e	BtsI-v2	rCutSmart	100	100	25	100	37°	No	A	λ DNA		1
R, e	BtsIMutI	rCutSmart	100	50	10	100	55°	80°	A	pUC19 DNA		b
TS	Cac8I	rCutSmart	50	75	100	100	37°	65°	B	λ DNA	CpG	b
R, TS	ClaI	rCutSmart	10	50	50	100	37°	65°	A	λ DNA (dam-)	dam, CpG	
R, TS, 2+site	CspCI	rCutSmart	10	100	10	100	37°	65°	A	λ DNA		e
R, TS	CviAII	rCutSmart	50	50	10	100	25°	65°	C	λ DNA		
R	CviKI-1	rCutSmart	25	100	100	100	37°	No	A	pBR322 DNA		1, b
R, TS	CviQI	r3.1	75	100*	100	75*	25°	No	C	λ DNA		b
R, TS	DdeI	rCutSmart	75	100	100	100	37°	65°	B	λ DNA		
R, TS	DpnI	rCutSmart	100	100	75	100	37°	80°	B	pBR322 DNA (dam methylated)	CpG	b
R, TS	DpnII	U	25	25	100*	25	37°	65°	B	λ DNA (dam-)	dam	
R, TS	DraI	rCutSmart	75	75	50	100	37°	65°	A	λ DNA		
R, TS, e	DraIII-HF	rCutSmart	<10	50	10	100	37°	No	B	λ DNA	CpG	b
R, TS	DrdI	rCutSmart	25	50	10	100	37°	65°	A	pUC19 DNA	CpG	3
R	EaeI	rCutSmart	10	50	<10	100	37°	65°	A	λ DNA	dcm, CpG	b
R, TS, e	EagI-HF	rCutSmart	25	100	100	100	37°	65°	B	pXba DNA	CpG	
R, TS	EarI	rCutSmart	50	10	<10	100	37°	65°	B	λ DNA	CpG	b, d
R	EciI	rCutSmart	100	50	50	100	37°	65°	A	λ DNA	CpG	2
R, TS	Eco53kI	rCutSmart	100	100	<10	100	37°	65°	A	pXba DNA	CpG	3, b
R, TS	EcoNI	rCutSmart	50	100	75	100	37°	65°	A	λ DNA		b
R, TS	EcoO109I	rCutSmart	50	100	50	100	37°	65°	A	λ DNA (HindIII digest)	dcm	3
R, TS, 2+site	EcoP15I	r3.1 + ATP	75	100	100	100	37°	65°	A	pUC19 DNA		e
R, TS	EcoRI	U	25	100*	50	50*	37°	65°	C	λ DNA	CpG	
R, TS, e	EcoRI-HF	rCutSmart	10	100	<10	100	37°	65°	C	λ DNA	CpG	
R, TS	EcoRV	r3.1	10	50	100	10	37°	80°	A	λ DNA	CpG	
R, TS, e	EcoRV-HF	rCutSmart	25	100	100	100	37°	65°	B	λ DNA	CpG	
R, TS	Esp3I	rCutSmart	100	100	<10	100	37°	65°	B	λ DNA	CpG	
R	FatI	r2.1	10	100	50	50	55°	80°	A	pUC19 DNA		
R	FauI	rCutSmart	100	50	10	100	55°	65°	A	λ DNA	CpG	3, b, d
R, TS	Fnu4HI	rCutSmart	<10	<10	<10	100	37°	No	A	λ DNA	CpG	a
R, 2+site	FokI	rCutSmart	100	100	75	100	37°	65°	A	λ DNA	dcm, CpG	3, b, d
R, TS	FseI	rCutSmart	100	75	<10	100	37°	65°	B	pBC4 DNA	dcm, CpG	
R	FspEI	rCutSmart + Enz. Activ.	<10	<10	<10	100	37°	80°	B	pBR322 (dcm+) DNA		1, e
R, TS	FspI	rCutSmart	10	100	10	100	37°	No	C	λ DNA	CpG	b
R, TS	HaeII	rCutSmart	25	100	10	100	37°	80°	A	λ DNA	CpG	
R, TS	HaeIII	rCutSmart	50	100	25	100	37°	80°	A	λ DNA		
R	HgaI	r1.1	100	100	25	100*	37°	65°	A	ΦX174 DNA	CpG	1
R, TS	HhaI	rCutSmart	25	100	100	100	37°	65°	A	λ DNA	CpG	
R, TS	HinP1I	rCutSmart	100	100	100	100	37°	65°	A	λ DNA	CpG	
R, TS	HincII	rCutSmart	25	100	100	100	37°	65°	B	λ DNA	CpG	
R	HindIII	r2.1	25	100	50	50	37°	80°	B	λ DNA		2
R, TS, e	HindIII-HF	rCutSmart	10	100	10	100	37°	80°	B	λ DNA		
R, TS	HinfI	rCutSmart	50	100	100	100	37°	80°	A	λ DNA	CpG	
R	HpaI	rCutSmart	<10	75*	25	100	37°	No	A	λ DNA	CpG	1
R, TS	HpaII	rCutSmart	100	50	<10	100	37°	80°	A	λ DNA	CpG	
R, TS	HphI	rCutSmart	50	50	<10	100	37°	65°	B	λ DNA	dcm	1, b, d
R, TS	Hpy166II	rCutSmart	100	100	50	100	37°	65°	C	pBR322 DNA	CpG	
R	Hpy188I	rCutSmart	25	100	50	100	37°	65°	A	pBR322 DNA	dam	1, b
R	Hpy188III	rCutSmart	100	100	10	100	37°	65°	B	pUC19 DNA	dam, CpG	3, b
R	Hpy99I	rCutSmart	50	10	<10	100	37°	65°	A	λ DNA	CpG	
R, TS	HpyAV	rCutSmart	100	100	25	100	37°	65°		λ DNA	CpG	3, b, d
R	HpyCH4III	rCutSmart	100	25	<10	100	37°	65°	A	λ DNA		b
R, TS	HpyCH4IV	rCutSmart	100	50	25	100	37°	65°	A	pUC19 DNA	CpG	
R, TS	HpyCH4V	rCutSmart	50	50	25	100	37°	65°	A	λ DNA		
R	I-CeuI	rCutSmart	10	10	10	100	37°	65°	B	pBHS ScaI-linearized Control Plasmid		
R	I-SceI	rCutSmart	10	50	25	100	37°	65°	B	pGPS2 NotI-linearized Control Plasmid		
R	KasI	rCutSmart	50	100	50	100	37°	65°	B	pBR322 DNA	CpG	3
R, TS, e	KpnI-HF	rCutSmart	100	25	<10	100	37°	No	A	pXba DNA		
R	LpnPI	rCutSmart + Enz. Activ.	<10	<10	<10	100	37°	65°	B	pBR322 (dcm+) DNA		1, e
R, TS	MboI	rCutSmart	75	100	100	100	37°	65°	A	λ DNA (dam-)	dam, CpG	
R, TS, 2+site	MboII	rCutSmart	100*	100	50	100	37°	65°	C	λ DNA (dam-)	dam	b
R, TS, e	MfeI-HF	rCutSmart	75	25	<10	100	37°	No	A	λ DNA		
R, TS	MluCI	rCutSmart	100	10	10	100	37°	No	A	λ DNA		
R, TS, e	MluI-HF	rCutSmart	25	100	100	100	37°	No	A	λ DNA	CpG	
R, TS	MlyI	rCutSmart	50	50	10	100	37°	65°	A	λ DNA		b, d
R, TS, 2+site	MmeI	rCutSmart	50	100	50	100	37°	65°	B	ΦX174 RF I DNA	CpG	b, c
R, TS	MnlI	rCutSmart	75	100	50	100	37°	65°	B	λ DNA		b
R	MscI	rCutSmart	25	100	100	100	37°	80°	C	λ DNA	dcm	
R, TS	MseI	rCutSmart	75	100	75	100	37°	65°	A	λ DNA		
R, TS	MslI	rCutSmart	50	50	<10	100	37°	80°	A	λ DNA		
R, TS	MspA1I	rCutSmart	10	50	10	100	37°	65°	B	λ DNA	CpG	
R, TS	MspI	rCutSmart	75	100	50	100	37°	No	A	λ DNA		
R	MspJI	rCutSmart + Enz. Activ.	<10	<10	<10	100	37°	65°	B	pBR322 (dcm+) DNA		1, e
R, TS	MwoI	rCutSmart	<10	100	100	100	60°	No	B	λ DNA	CpG	
R, 2+site	NaeI	rCutSmart	25	25	<10	100	37°	No	A	pXba DNA	CpG	b
R, 2+site	NarI	rCutSmart	100	100	10	100	37°	65°	A	pXba DNA	CpG	
R	Nb.BbvCI	rCutSmart	25	100	100	100	37°	80°	A	supercoiled plasmid DNA		e
R	Nb.BsmI	r3.1	<10	50	100	10	65°	80°	A	supercoiled plasmid pBR322 DNA		e
R	Nb.BsrDI	rCutSmart	25	100	100	100	65°	80°	A	supercoiled pUC19 DNA		e
R	Nb.BssSI	r3.1	10	100	100	25	37°	No	B	supercoiled pUC19 DNA		e
R	Nb.BtsI	rCutSmart	75	100	75	100	37°	80°	A	supercoiled pUC101 DNA (dam-/dcm-)		e
R, TS	NciI	rCutSmart	100	25	10	100	37°	No	A	λ DNA	CpG	b
R, TS	NcoI	r3.1	100	100	100	100	37°	80°	A	λ DNA		
R, TS, e	NcoI-HF	rCutSmart	50	100	10	100	37°	80°	B	λ DNA		
R, TS	NdeI	rCutSmart	75	100	100	100	37°	65°	A	λ DNA		
R, TS, 2+site	NgoMIV	rCutSmart	100	50	10	100	37°	No	A	pXba DNA	CpG	1
R, TS, e	NheI-HF	rCutSmart	100	25	10	100	37°	80°	C	λ DNA (HindIII digest)	CpG	
R, TS	NlaIII	rCutSmart	<10	<10	<10	100	37°	65°	B	ΦX174 RF I DNA		
R	NlaIV	rCutSmart	10	10	10	100	37°	65°	B	pBR322 DNA	dcm, CpG	
R, 2+site	NmeAIII	rCutSmart	10	10	<10	100	37°	65°	B	ΦX174 RF I DNA		c
R, TS	NotI	r3.1	<10	50	100	25	37°	65°	C	pBC4 DNA	CpG	
R, TS, e	NotI-HF	rCutSmart	25	100	25	100	37°	65°	A	pBC4 DNA	CpG	
R, TS, e	NruI-HF	rCutSmart	0	25	50	100	37°	No	A	λ DNA	dam, CpG	
R, TS	NsiI	r3.1	10	75	100	25	37°	65°	B	λ DNA		
R, TS, e	NsiI-HF	rCutSmart	<10	20	<10	100	37°	80°	B	λ DNA		
R, TS	NspI	rCutSmart	100	100	<10	100	37°	65°	A	λ DNA		
R	Nt.AlwI	rCutSmart	10	100	100	100	37°	80°	A	pUC101 DNA (dam-/dcm-)	dam	e
R	Nt.BbvCI	rCutSmart	50	100	10	100	37°	80°	A	supercoiled plasmid DNA	CpG	e
R	Nt.BsmAI	rCutSmart	100	50	10	100	37°	65°	A	supercoiled plasmid DNA	CpG	e
R	Nt.BspQI	r3.1	<10	25	100	10	50°	80°	B	supercoiled pUC19 DNA		e
R	Nt.BstNBI	r3.1	0	10	100	10	55°	80°	A	T7 DNA		e
R	Nt.CviPII	rCutSmart	10	100	25	100	37°	65°	A	pUC19 DNA	CpG	e
R	PI-PspI	U + rAlbumin	10	10	10	10	65°	No	B	pAKR7 XmnI-linearized Control Plasmid		
R	PI-SceI	U + rAlbumin	10	10	10	10	37°	65°	B	pBSvdeX XmnI-linearized Control Plasmid		
R, TS	PacI	rCutSmart	100	75	10	100	37°	65°	A	pNEB193 DNA		
R, TS	PaeR7I	rCutSmart	25	100	10	100	37°	No	A	λ DNA (HindIII digest)	CpG	
R, 2+site	PaqCI	rCutSmart PaqCI Activator	10	100	10	100	37°	65°	B	λ DNA	CpG	1
R	PciI	r3.1	50	75	100	50*	37°	80°	B	pXba DNA		
R, TS	PflFI	rCutSmart	25	100	25	100	37°	65°	A	pBC4 DNA		b
R, TS	PflMI	r3.1	0	100	100	50	37°	65°	A	λ DNA	dcm	3, b, d
R, 2+site	PleI	rCutSmart	25	50	25	100	37°	65°	A	λ DNA	CpG	b, d
R, 2+site	PluTI	rCutSmart	100	25	<10	100	37°	65°	A	pXba DNA	CpG	b
R, TS	PmeI	rCutSmart	<10	50	10	100	37°	65°	A	λ DNA	CpG	
R, TS	PmlI	rCutSmart	100	50	<10	100	37°	65°	A	λ DNA (HindIII digest) DNA	CpG	
R, TS	PpuMI	rCutSmart	<10	<10	<10	100	37°	No	B	λ DNA (HindIII digest)	dcm	
R, TS	PshAI	rCutSmart	25	50	10	100	37°	65°	A	λ DNA	CpG	
R, TS, e	PsiI-v2	rCutSmart	25	50	10	100	37°	65°	B	λ DNA		3
R	PspGI	rCutSmart	25	100	50	100	75°	No	A	T7 DNA	dcm	3
R	PspOMI	rCutSmart	10	10	<10	100	37°	65°	B	pXba DNA	dcm, CpG	
R	PspXI	rCutSmart	<10	100	25	100	37°	No	B	λ DNA (HindIII digest)	CpG	
R, TS	PstI	r3.1	75	75	100	50*	37°	80°	C	λ DNA		
R, TS, e	PstI-HF	rCutSmart	10	75	50	100	37°	No	C	λ DNA		
R, TS, e	PvuI-HF	rCutSmart	25	100	100	100	37°	No	B	pXba DNA	CpG	
R, TS	PvuII	r3.1	50	100	100	100*	37°	No	B	λ DNA		
R, TS, e	PvuII-HF	rCutSmart	<10	<10	<10	100	37°	No	B	λ DNA		
R, TS	RsaI	rCutSmart	25	50	<10	100	37°	No	A	λ DNA	CpG	
R, 2+site	RsrII	rCutSmart	25	75	10	100	37°	65°	C	λ DNA	CpG	
R, TS, e	SacI-HF	rCutSmart	10	50	<10	100	37°	65°	A	λ DNA (HindIII digest)	CpG	
R, TS, 2+site	SacII	rCutSmart	10	100	10	100	37°	65°	A	pXba DNA	CpG	
R, TS	SalI	r3.1	<10	<10	100	<10	37°	65°	A	λ DNA (HindIII digest)	CpG	
R, TS, e	SalI-HF	rCutSmart	10	100	100	100	37°	65°	A	λ DNA (HindIII digest)	CpG	
R, TS	SapI	rCutSmart	75	50	<10	100	37°	65°	B	λ DNA		
R	Sau3AI	r1.1	100	50	10	100	37°	65°	A	λ DNA	CpG	b
R	Sau96I	rCutSmart	50	100	100	100	37°	65°	A	λ DNA	dcm, CpG	
R, TS, e	SbfI-HF	rCutSmart	50	25	<10	100	37°	80°	B	λ DNA		
R, TS, e	ScaI-HF	rCutSmart	100	100	10	100	37°	80°	B	λ DNA		
R	ScrFI	rCutSmart	100	100	100	100	37°	65°	C	λ DNA	dcm, CpG	2, a
R	SexAI	rCutSmart	100	75	50	100	37°	65°	A	pBC4 DNA (dcm-)	dcm	3, b, d
R	SfaNI	r3.1	<10	75	100	25	37°	65°	B	ΦX174 RF I DNA	CpG	3, b
R	SfcI	rCutSmart	75	50	25	100	37°	65°	B	λ DNA		3
R, TS, 2+site	SfiI	rCutSmart	25	100	50	100	50°	No	C	pXba DNA	dcm, CpG	
R, TS	SfoI	rCutSmart	50	100	100	100	37°	No	B	λ DNA (HindIII digest)	dcm, CpG	
R, 2+site	SgrAI	rCutSmart	100	100	10	100	37°	65°	A	λ DNA	CpG	1
R, TS	SmaI	rCutSmart	<10	<10	<10	100	37°	65°	B	λ DNA (HindIII digest)	CpG	b
R	SmlI	rCutSmart	25	75	25	100	55°	No	A	λ DNA		b
R	SnaBI	rCutSmart	50*	50	10	100	37°	80°	A	T7 DNA	CpG	1
R, TS, e	SpeI-HF	rCutSmart	25	50	10	100	37°	80°	C	pXba-XbaI DNA		
R	SphI	r2.1	100	100	50	100	37°	65°	B	λ DNA		2
R, TS, e	SphI-HF	rCutSmart	50	25	10	100	37°	65°	B	λ DNA		
R, TS	SrfI	rCutSmart	10	50	0	100	37°	65°	B	pNEB193-SrfI DNA	CpG	
R, TS, e	SspI-HF	rCutSmart	25	100	<10	100	37°	65°	B	λ DNA		
R, TS	StuI	rCutSmart	50	100	50	100	37°	No	A	λ DNA	dcm	
R, TS	StyD4I	rCutSmart	10	100	100	100	37°	65°	B	λ DNA	dcm, CpG	
R, TS, e	StyI-HF	rCutSmart	25	100	25	100	37°	65°	A	λ DNA		
R, TS	SwaI	r3.1	10	10	100	10	25°	65°	B	pXba DNA		b, d
R, TS, e	TaqI-v2	rCutSmart	50	100	50	100	65°	No	B	λ DNA	dam	
R, TS	TfiI	rCutSmart	50	100	100	100	65°	No	C	λ DNA	CpG	
R, TS	TseI	rCutSmart	75	100	100	100	65°	No	B	λ DNA	CpG	3
R	Tsp45I	rCutSmart	100	50	<10	100	65°	No	A	λ DNA		
TS	TspMI	rCutSmart	50*	75*	50*	100	75°	No	B	pBC4 DNA	CpG	d
R, TS	TspRI	rCutSmart	25	50	25	100	65°	No	B	λ DNA		
R, TS	Tth111I	rCutSmart	25	100	25	100	65°	No	B	pBC4 DNA		b
R	WarmStart Nt.BstNBI	r3.1	0	10	100	25	55°	80°	A	T7 DNA		
R, TS	XbaI	rCutSmart	<10	100	75	100	37°	65°	A	λ DNA (dam-/Hind III digest)	dam	
R	XcmI	r2.1	10	100	25	100*	37°	65°	C	λ DNA		2
R, TS	XhoI	rCutSmart	75	100	100	100	37°	65°	A	λ DNA (HindIII digest)	CpG	b
R, TS	XmaI	rCutSmart	25	50	<10	100	37°	65°	A	pXba DNA	CpG	3
R, TS	XmnI	rCutSmart	50	75	<10	100	37°	65°	A	λ DNA		b
R	ZraI	rCutSmart	100	25	10	100	37°	80°	B	λ DNA	CpG	

Single Letter Codes:
B = C or G or T; D = A or G or T; H = A or C or T; K = G or T; M = A or C; N = A or C or G or T; R = A or G; S = C or G; V = A or C or G; W = A or T; Y = C or T

Chart Legend and Notes:

- Recombinant
- Time-Saver qualified (cleaves substrate in 5 – 15 min under recommended conditions)
- Engineered for maximum performance
- dam — dam methylation sensitivity
- dcm — dcm methylation sensitivity
- CpG — CpG methylation sensitivity (applies to eukaryotic genomic DNA only)
- 2*site — Indicates that the restriction enzyme requires two or more sites for cleavage
- **U** Supplied with its own unique reaction buffer that is different from the four standard NEBuffers. Compatibility with the four standard NEBuffers is indicated by the chart.
- **SAM/ATP** Supplied with a separate vial of S-adenosylmethionine (SAM) or Adenosine 5´-Triphosphate (ATP). To obtain 100% activity, SAM or ATP should be added to the 1X reaction mix as specified on the product data card.

The following notes appear with any enzymes having ligation efficiencies lower than 100% as assessed by ligation and recutting:

a. Ligation is < 10%
b. Ligation is 25% – 75%
c. Recutting after ligation is < 5%
d. Recutting after ligation is 50% – 75%
e. Ligation and recutting after ligation is not applicable since the enzyme is either a nicking enzyme, is affected by methylation, or if the enzyme cleaves outside its recognition sequence.

The following notes appear with any enzymes when star activity is a concern:

1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.
2. Star activity may result from extended digestion.
3. Star activity may result from a glycerol concentration of > 5%.
* May exhibit star activity in this buffer

+For added flexibility, NEB offers an isoschizomer or HF enzyme, supplied with rCutSmart Buffer.

To address the need for BSA-free reagents, NEB has switched our BSA-containing reaction buffers to Recombinant Albumin (rAlbumin)-containing buffers. We are also in the process of transitioning our enzyme formulations to contain rAlbumin. NEB has rigorously tested these changes and has not seen a difference in performance with these changes. Learn more at **www.neb.com/BSA-free**.

NEBuffer Compositions (1X)

- NEBuffer r1.1: 10 mM Bis Tris Propane-HCl, 10 mM $MgCl_2$, 100 µg/ml Recombinant Albumin (pH 7.0 @ 25°C)
- NEBuffer r2.1: 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 100 µg/ml Recombinant Albumin (pH 7.9 @ 25°C)
- NEBuffer r3.1: 100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 µg/ml Recombinant Albumin (pH 7.9 @ 25°C)
- rCutSmart Buffer: 50 mM Potassium acetate, 20 mM Tris-acetate, 10 mM Magnesium acetate, 100 µg/ml Recombinant Albumin (pH 7.9 @ 25°C)

Double Digest Finder · NEBcutter® · NEBioCalculator® · NEBcloner® · Enzyme Finder · REBASE® and more...

To benefit from our interactive NEB online tools & resources, please visit **www.neb.com/nebtools**

Activity of DNA Modifying Enzymes in rCutSmart Buffer

A selection of DNA modifying enzymes were assayed in rCutSmart Buffer and functional activity was compared to the activity in their supplied buffers. Reactions were set up according to the recommended reaction conditions, with rCutSmart Buffer (plus required supplement) replacing the supplied buffer.

Tech Tip: When supplements are required, one can simply add the supplied buffer of the respective modifying enzyme at 1X concentration to the rCutSmart Buffer to achieve appropriate activity for most applications – no change of buffers needed.

Enzyme	Activity in rCutSmart	Required Supplements
Phosphatases:		
Antarctic Phosphatase	+++	Requires Zn^{2+}
Shrimp Alkaline Phosphatase (rSAP)	+++	
Quick CIP	+++	
Ligases:		
T4 DNA Ligase	+++	Requires ATP
Hi-T4™ DNA Ligase	+++	Requires ATP
Salt-T4® DNA Ligase	+	Requires ATP
E. coli DNA Ligase	+++	Requires NAD
T3 DNA Ligase	+++	Requires ATP + PEG
T7 DNA Ligase	+++	Requires ATP + PEG
Polymerases:		
T4 DNA Polymerase	+++	
DNA Polymerase I, Large (Klenow) Fragment	+++	
DNA Polymerase I	+++	
DNA Polymerase Klenow Exo⁻	+++	
Bst DNA Polymerase	+++	
phi29 DNA Polymerase	+++	Requires DTT
T7 DNA Polymerase (unmodified)	+++	
Transferases/Kinases:		
T4 Polynucleotide Kinase	+++	Requires ATP + DTT
T4 PNK (3´ phosphatase minus)	+++	Requires ATP + DTT
CpG Methyltransferase (M. SssI)	+++	
GpC Methyltransferase (M. CviPI)	+	Requires DTT
T4 Phage β-glucosyltransferase (T4-BGT)	+++	

Enzyme	Activity in rCutSmart	Required Supplements
Nucleases, other:		
DNase I (RNase-free)	+++	Requires Ca^{2+}
DNase I-XT	+++	Requires Ca^{2+}
Endonuclease III (Nth)	+++	
Endonuclease VIII	+++	
Exonuclease VII	+++	
Exonuclease V (Rec BCD)	+++	Requires ATP
Exonuclease III	+++	
Exonuclease I	+++	
FPG	+++	
Lambda Exonuclease	++	
McrBC	+++	
Micrococcal Nuclease	+++	Requires Ca^{2+}
$RecJ_f$	+++	
T5 Exonuclease	+++	
T7 Exonuclease	+++	
Thermolabile Exo I	+++	
Thermolabile USER II	+++	
Thermostable USER III	++	
Thermostable OGG	+++	
USER™ Enzyme	+++	

+++ full functional activity
++ 50–100% functional activity
+ 0–50% functional activity

Updates to this chart can be found at **NEBCutSmart.com**