

SphI

Sequence of SphI: 5'...GCATGC...3' 3'...CGTACG...5'

Catalog-no	Description	units
250-137S	SphI	250 u
250-137L	SphI	5x250 u

Overhang: 5' - **CATG**

Cut Site:

GCATG/C
C/GTACG

Isoschizomers: *Bbu* I, *Pae* I, *Spa*H I

Neoschizomers: N/A

Source: *Streptomyces phaeochromogenes*.

Buffer supplied: 10x M .

Substrate for unit definition: λ DNA.

Reaction conditions: 50 mM NaCl, 10 mM Tris-HCl (pH 7.9 at 25°C), 10 mM MgCl₂, 1 mM dithiothreitol, 100 μ g/ml BSA. **Incubate at 37°C.**

Storage buffer: 100 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 400 μ g/ml BSA and 50% glycerol.

Store: at -20°C.

Absence of contaminants: Fifty units of SphI do not produce any unspecific cleavage products after 16 hrs incubation with 1 μ g of λ DNA at 37°C. After ten-fold overdigestion with SphI, more than 98% of the DNA fragments can be ligated and recut with this enzyme.

Heat inactivation: 65°C for 20 minutes.

Recommended Reaction Conditions

* Requires Triton X-100 for optimal activity.

Restriction Enzyme	Tris-HCl (Tris-Acetate) {Bis Tris Propane-HCl}		NaCl (K-Acetate) {KCl}	MgCl ₂ (Mg-Acetate)	DTT	BSA	TX-100	Temp.	Buffer
	mM	pH (25°C)	mM	mM	mM	µg/ml	%	°C	
<i>Alu</i> I	10	7.9	-	10	1	100	-	37	L
<i>Apa</i> L I	10	7.9	-	10	1	100	-	37	L
<i>Asu</i> II	10	7.9	50	10	1	100	0.1	37	M*
<i>Bam</i> H I	10	7.9	100	5	1	100	-	37	U
<i>Bcl</i> I	10	7.9	50	10	1	100	-	50	M
<i>Bgl</i> I	100	7.9	50	5	-	100	0.025	37	U
<i>Bgl</i> II	50	7.9	100	10	1	100	-	37	H
<i>Bse</i> A I	10	8.0	100	5	1	100	0.02	55	U
<i>Bse</i> B I	10	7.9	50	10	1	100	-	60	M
<i>Bse</i> C I	50	7.9	100	10	1	100	-	55	H
<i>Bsh</i> F I	(20)	7.9	(50)	(10)	1	100	-	37	A
<i>Bsi</i> S I	(33)	7.9	(66)	(10)	0.5	100	0.1	55	U
<i>Bss</i> A I	20	8.5	{100}	3	-	100	0.04	65	U
<i>Bst</i> E I	10	7.4	{100}	5	1	100	0.1	60	U
<i>Csp</i> A I	{10}	7.0	-	10	1	100	-	37	U
<i>Eco</i> R I	100	7.4	50	5	-	100	0.025	37	U
<i>Eco</i> R V	10	7.9	50	10	1	100	-	37	M
<i>Hind</i> III	10	7.9	50	10	1	100	-	37	M
<i>Hinf</i> I	50	7.9	100	10	1	100	-	37	H
<i>Hpa</i> I	(20)	7.9	(50)	(10)	1	100	-	37	A
<i>Kpn</i> I	10	7.0	-	10	1	100	0.01	37	U
<i>Mbo</i> I	10	8.0	{100}	10	1	100	-	37	U
<i>Msp</i> C I	10	7.9	150	10	1	100	-	37	SH
<i>Nae</i> I	10	7.9	-	10	1	100	-	37	L
<i>Nco</i> I	50	7.9	100	10	1	100	0.02	37	H*
<i>Nhe</i> I	(20)	7.9	(50)	(10)	1	100	-	37	A
<i>Not</i> I	50	7.9	100	5	1	100	-	37	U
<i>Nru</i> I	50	8.0	{100}	10	-	100	-	37	U
<i>Psp</i> P I	10	7.9	50	10	1	100	-	25	M
<i>Pst</i> I	50	7.4	100	10	1	100	-	37	U
<i>Pvu</i> II	10	7.9	50	10	1	100	-	37	M
<i>Rsa</i> I	10	7.9	50	10	1	100	-	37	M
<i>Sal</i> I	10	7.9	150	10	1	100	-	37	SH
<i>Sca</i> I	10	7.4	100	10	1	100	-	37	U
<i>Sfi</i> I	10	7.9	50	10	1	100	-	50	M
<i>Sgr</i> B I	10	7.9	-	10	1	100	0.1	37	L*
<i>Sla</i> I	10	7.9	150	10	1	100	-	37	SH
<i>Sma</i> I	(20)	7.9	(50)	(10)	1	100	-	25	A
<i>Sna</i> B I	{10}	7.0	-	10	1	100	-	37	U
<i>Sph</i> I	10	7.9	50	10	1	100	-	37	M
<i>Sse</i> B I	50	7.9	100	10	1	100	-	37	H
<i>Ssp</i> I	50	7.9	100	10	1	100	-	37	H
<i>Sst</i> I	10	7.9	-	10	1	100	-	37	L
<i>Sty</i> I	50	7.9	100	10	1	100	-	37	H
<i>Taq</i> I	20	8.5	{100}	3	-	100	0.04	65	U
<i>Xba</i> I	10	7.9	50	10	1	100	-	37	M <i>Sau</i> 3AI
	10	7.9	50	10	1	100	-	37	M

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Relative Activity of Restriction Enzymes in Reactions Buffers

This table lists relative activities of each restriction enzyme with each buffer assuming the activity of the enzyme under optimal conditions to be 100%.

Restriction enzyme	Recommended buffer	Enzyme activity (%)				
		L	M	H	SH	A
<i>Alu I</i>	L	100	100	75	10-25	75
<i>ApaI</i>	L	100	100	10	<10	10-25
<i>Asu II</i>	M*	75	100	50-75	25	50
<i>BamH I</i>	U	75	75-100	100	50-75	75
<i>Bcl I</i> (50°C)	M	10-25	100	75	50-75	10-25
<i>Bgl I</i>	U	10-25	75-100	75-100	75-100	50
<i>Bgl II</i>	H	10	75	100	75-100	10
<i>BseA I</i> (55°C)	U	10	50	75-100	50-75	10
<i>BseB I</i> (60°C)	M	10-25	100	50	25-50	<10
<i>BseC I</i> (55°C)	H	10	50	100	75-100	50
<i>BshF I</i>	A	50-75	75-100	75	50-75	100
<i>BsiS I</i> (55°C)	U	25	50	25	10-25	100
<i>BssA I</i> (65°C)	U	10	25	75	50	25
<i>CspA I</i>	U	50	<10	<10	<10	<10
<i>EcoR I</i>	U	25-50	50-75	75	50-75	75
<i>EcoR V</i>	M	10-25	100	50	<10	75
<i>Hind III</i>	M	25-50	100	10-25	10-25	50
<i>BstE II</i> (60°C)	U	50	50-75	75-100	50	75
<i>Hinf I</i>	H	10-25	50	100	75-100	50
<i>Hpa I</i>	A	25-50	10-25	10-25	10-25	100
<i>Kpn I</i>	U	75-100	25-50	<10	<10	50
<i>Mbo I</i>	U	50-100	50-100	50-100	50	50-100
<i>MspC I</i>	SH	<10	25-50	75-100	100	50
<i>Nae I</i>	L	100	25-50	25	<10	50
<i>Nco I</i>	H*	50-75	75-100	100	100	75
<i>Nhe I</i>	A	100	50-75	0-20	<10	100
<i>Not I</i>	U	<10	25-50	75-100	75	50
<i>Nru I</i>	U	<10	<10	75	50-75	10
<i>PspP I</i> (25°C)	M	50-75	100	50	25-50	10
<i>Pst I</i>	U	10-25	50-75	75-100	50-75	50
<i>Pvu II</i>	M	25-50	100	100	25-50	50
<i>Rsa I</i>	M	75-100	100	50	<10	<10
<i>Sal I</i>	SH	<10	25-50	50	100	<10
<i>Sau3AI</i>	M	50	100	50	<10	50
<i>Sca I</i>	U	<10	50-75	100	75-100	25
<i>Sfi I</i> (50°C)	M	75-100	100	25-50	10-25	75-100
<i>SgrB I</i>	L*	75-100	75	50-75	25-50	<10
<i>Sla I</i>	SH	25-50	75	75-100	100	10-25
<i>Sma I</i> (25°C)	A	<10	<10	<10	<10	100
<i>SnaB I</i>	U	50-75	50	25	<10	100
<i>Sph I</i>	M	75-100	100	50	50	50
<i>SseB I</i>	H	50-75	75-100	100	50-75	50
<i>Ssp I</i>	H	10-25	50-75	100	75-100	50
<i>Sst I</i>	L	100	25-50	25	<10	50
<i>Sty I</i>	H	25-50	75-100	100	75-100	<10
<i>Taq I</i> (65°C)	U	10-25	50-75	75-100	50-75	50
<i>Xba I</i>	M	50-75	100	75	75	75

- Reactions were carried out at 37°C except for *Bcl I*, *BseA I*, *BseB I*, *BseC I*, *BsiS I*, *BssA I*, *BstE II*, *PspP I*, *Sfi I*, *Sma I* and *Taq I*. The reaction temperature for these enzymes is indicated in parenthesis.
- All reactions were carried out in the presence of BSA, 100µg/ml.

Suggested Buffers for Double Digestion

	<i>Bam</i> HI	<i>Bgl</i> II	<i>Eco</i> RI	<i>Eco</i> RV	<i>Hind</i> III	<i>Kpn</i> I	<i>Nco</i> I	<i>Nhe</i> I	<i>Not</i> I	<i>Pst</i> I	<i>Pvu</i> II	<i>Sal</i> I	<i>Sgr</i> BI	<i>Sla</i> I	<i>Sma</i> I	<i>Sph</i> I	<i>Sst</i> I	
	U	H	U	M	M	U	H ⁺	A	U	U	M	SH	L	SH	A	M	L	
<i>Bgl</i> II	H	H																
<i>Eco</i> RI	U		<i>Eco</i> RI	<i>Eco</i> RI														
<i>Eco</i> RV	M	M	M	<i>Eco</i> RI														
<i>Hind</i> III	M	M	M	<i>Eco</i> RI	M													
<i>Kpn</i> I	U	seq	M	seq	M	<i>Kpn</i> I/M												
<i>Nco</i> I	H ⁺	<i>Bam</i> HI	H	<i>Eco</i> RI	M	M	L											
<i>Nhe</i> I	A	A	M	A	A	M	L	A										
<i>Not</i> I	U	<i>Bam</i> HI	H	<i>Eco</i> RI	H	M	seq	SH	A									
<i>Pst</i> I	U	<i>Bam</i> HI	H	<i>Eco</i> RI	M/H	M	seq	H	A	H								
<i>Pvu</i> II	M	<i>Bam</i> HI	H	<i>Eco</i> RI	M	M	A	H	M	H	H							
<i>Sal</i> I	SH	SH	SH	<i>Eco</i> RI	H	seq	seq	SH	seq	SH	SH	H						
<i>Sgr</i> BI	L	L/M	M	M/H	M	M	L	M	L	seq	H	M	seq					
<i>Sla</i> I	SH	<i>Bam</i> HI	H	<i>Eco</i> RI	M	M	seq	SH	M	SH	H	H	SH	M/H				
<i>Sma</i> I	A	A	seq	seq	A	A	A	A	A	A	A	A	seq	seq	seq			
<i>Sph</i> I	M	M	M	<i>Eco</i> RI	M	M	L	M	L	<i>Not</i> I	M	M	SH	L	M	A		
<i>Sst</i> I	L	L	M	seq	M	M	L	L	L	A	A	A	seq	L	seq	A	L	
<i>Xba</i> I	M	M	M/H	<i>Eco</i> RI	M	M	L	M	A	<i>Not</i> I	H	M	SH	M	M/SH	A	M	L

Notes:

• All the reactions were carried out in the presence of BSA (100µg/ml). Our experience indicates that it is important to use BSA in reaction mixtures in order to obtain successful digestions of DNA. The presence of BSA gives complete and reproducible cleavages for a range of DNA substrates. BSA stabilizes the enzymes when digestions are performed for more than one hour at 37°C, since many restriction endonucleases in reaction buffers without BSA can survive at this temperature for 10-20 minutes only or even less. Also, BSA binds metal ions, and other chemicals, which might be present in buffers or DNA preparations, thereby inactivating restriction endonucleases. The following enzymes can exhibit "star" activity: *Bam*H I, *Bcl* I, *Bse*B I, *Bss*A I, *Eco*R I, *Eco*R V, *Hind* III, *Hpa* I, *Kpn* I, *Nco* I, *Nru* I, *Pst* I, *Pvu* II, *Sal* I, *Sca* I, *Sna*B I, *Sph* I, *Ssp* I, *Xba* I.