Recommended Reaction Conditions for Minotech Restriction Endonucleases

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	
Alu I	10	7.9	-	10	1	100	-	37	L
*Apa*L I	10	7.9	-	10	1	100	-	37	L
Asu II	10	7.9	50	10	1	100	0.1	37	M*
*Bam*H I	10	7.9	100	5	1	100	-	37	U
Bcl I	10	7.9	50	10	1	100	-	50	M
Bgl I	100	7.9	50	5	-	100	0.025	37	U
Bgl II	50	7.9	100	10	1	100	-	37	H
*Bse*A I	10	8.0	100	5	1	100	0.02	55	U
*Bse*B I	10	7.9	50	10	1	100	-	60	M
*Bse*C I	50	7.9	100	10	1	100	-	55	H
*Bsh*F I	(20)	7.9	(50)	(10)	1	100	-	37	A
*Bsi*S I	(33)	7.9	(66)	(10)	0.5	100	0.1	55	U
*Bss*A I	20	8.5	{100}	3	-	100	0.04	65	U
*Bst*EI I	10	7.4	{100}	5	1	100	0.1	60	U
*Csp*AI	{10}	7.0	-	10	1	100	-	37	U
*Eco*R I	100	7.4	50	5	-	100	0.025	37	U
*Eco*R V	10	7.9	50	10	1	100	-	37	M
Hind III	10	7.9	50	10	1	100	-	37	M
Hinf I	50	7.9	100	10	1	100	-	37	H
Hpa I	(20)	7.9	(50)	(10)	1	100	-	37	A
Kpn I	10	7.0	-	10	1	100	0.01	37	U
*Mbo*I	10	8.0	{100}	10	1	100	-	37	U
*Msp*C I	10	7.9	150	10	1	100	-	37	SH
Nae I	10	7.9	-	10	1	100	-	37	L
Nco I	50	7.9	100	10	1	100	0.02	37	H*
Nhe I	(20)	7.9	(50)	(10)	1	100	-	37	A
Not I	50	7.9	100	5	1	100	-	37	U
Nru I	50	8.0	{100}	10	-	100	-	37	U
*Psp*P I	10	7.9	50	10	1	100		25	M
Pst I	50	7.4	100	10	1	100	-	37	U
Pvu II	10	7.9	50	10	1	100	-	37	M
Rsa I	10	7.9	50	10	1	100	-	37	M
Sal I	10	7.9	150	10	1	100	-	37	SH
Sca I	10	7.4	100	10	1	100	-	37	U
Sfi I	10	7.9	50	10	1	100	-	50	M
*Sgr*B I	10	7.9	-	10	1	100	0.1	37	L*
Sla I	10	7.9	150	10	1	100	-	37	SH
Sma I	(20)	7.9	(50)	(10)	1	100	-	25	A
*Sna*B I	{10}	7.0	-	10	1	100	-	37	U
Sph I	10	7.9	50	10	1	100	-	37	M
*Sse*B I	50	7.9	100	10	1	100	-	37	H
Ssp I	50	7.9	100	10	1	100	-	37	H
Sst I	10	7.9	-	10	1	100	-	37	L
Sty I	50	7.9	100	10	1	100	-	37	H
Taq I	20	8.5	{100}	3	-	100	0.04	65	U
Xba I	10	7.9	50	10	1	100	-	37	M
*Sau*3AI	10	7.9	50	10	1	100	-	37	M

* Requires Triton X-100 for optimal activity.

Relative Activity of Restriction Enzymes with Minotech 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	K
***Alu* I**	**L**	**100**	100	75	10-25	75	**100**
***Apa*L I**	**L**	**100**	100	10	<10	10-25	**100**
***Asu* II**	**U**	75	**100**	50-75	25	50	**100**
***Bam*H I**	**U**	75	75-100	**100**	50-75	75	**100**
***Bcl* I** (50°C)	**M**	10-25	**100**	75	50-75	10-25	**100**
***Bgl* I**	**U**	10-25	75-100	75-100	75-100	50	**100**
***Bgl* II**	**H**	10	75	**100**	75-100	10	**100**
***Bse*A I** (55°C)	**U**	10	50	75-100	50-75	10	**100**
***Bse*B I** (60°C)	**M**	10-25	**100**	50	25-50	<10	50
***Bse*C I** (55°C)	**H**	10	50	**100**	75-100	50	**100**
***Bsh*F I**	**A**	50-75	75-100	75	50-75	**100**	**100**
***Bsi*S I** (55°C)	**U**	25	50	25	10-25	**100**	**100**
***Bss*A I** (65°C)	**U**	10	25	75	50	25	**100**
***Csp*A I**	**U**	50	<10	<10	<10	<10	**100**
***Eco*R I**	**U**	25-50	50-75	75	50-75	75	**100**
***Eco*R V**	**M**	10-25	**100**	50	<10	75	**100**
***Hind* III**	**M**	25-50	**100**	10-25	10-25	50	**100**
***Bst*E II** (60°C)	**U**	50	50-75	75-100	50	75	**100**
***Hinf* I**	**H**	10-25	50	**100**	75-100	50	**100**
***Hpa* I**	**A**	25-50	10-25	10-25	10-25	**100**	**100**
***Kpn* I**	**U**	75-100	25-50	<10	<10	50	**100**
***Mbo* I**	**U**	50-100	50-100	50-100	50	50-100	**100**
***Msp*C I**	**SH**	<10	25-50	75-100	**100**	50	**100**
***Nae* I**	**L**	**100**	25-50	25	<10	50	**100**
***Nco* I**	**U**	50-75	75-100	**100**	**100**	75	**100**
***Nhe* I**	**A**	100	50-75	0-20	<10	**100**	**100**
***Not* I**	**U**	<10	25-50	75-100	75	50	50
***Nru* I**	**U**	<10	<10	75	50-75	10	**100**
***Psp*P I** (25°C)	**M**	50-75	**100**	50	25-50	10	**100**
***Pst* I**	**U**	10-25	50-75	75-100	50-75	50	**100**
***Pvu* II**	**M**	25-50	**100**	100	25-50	50	**100**
***Rsa* I**	**M**	75-100	**100**	50	<10	<10	**100**
***Sal* I**	**SH**	<10	25-50	50	**100**	<10	50
***Sau*3AI**	**M**	50	**100**	50	<10	50	**100**
***Sca* I**	**U**	<10	50-75	**100**	75-100	25	50
***Sfi* I** (50°C)	**M**	75-100	**100**	25-50	10-25	75-100	**100**
***Sgr*B I**	**U**	75-100	75	50-75	25-50	<10	**100**
***Sla* I**	**SH**	25-50	75	75-100	**100**	10-25	**100**
***Sma* I** (25°C)	**A**	<10	<10	<10	<10	**100**	**100**
***Sna*B I**	**U**	50-75	50	25	<10	**100**	**100**
***Sph* I**	**M**	75-100	**100**	50	50	50	**100**
***Sse*B I**	**H**	50-75	75-100	**100**	50-75	50	**100**
***Ssp* I**	**H**	10-25	50-75	**100**	75-100	50	**100**
***Sst* I**	**L**	**100**	25-50	25	<10	50	**100**
***Sty* I**	**H**	25-50	75-100	**100**	75-100	<10	50
***Taq* I** (65°C)	**U**	10-25	50-75	75-100	50-75	50	50
***Xba* I**	**M**	50-75	**100**	75	75	75	**100**

- Reactions were carried out at 37°C except for ***Bcl* I**, ***Bse*A I**, ***Bse*B I**, ***Bse*C I**, ***Bsi*S I**, ***Bss*A I**, ***Bst*E II**, ***Psp*P 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 Minotech Buffers for Double Digestion

		***Bam*HI**	***Bgl*II**	***Eco*RI**	***Eco*RV**	***Hind*III**	***Kpn*I**	***Nco*I**	***Nhe*I**	***Not*I**	***Pst*I**	***Pvu*II**	***Sal*I**	***Sgr*BI**	**SlaI**	***Sma*I**	***Sph*I**	***Sst*I**
		U	**H**	**U**	**M**	**M**	**U**	**H$^+$**	**A**	**U**	**U**	**M**	**SH**	**L**	**SH**	**A**	**M**	**L**
***Bgl*II**	**H**	H																
***Eco*RI**	**U**	*Eco*RI	*Eco*RI															
***Eco*RV**	**M**	M	M	*Eco*RI														
***Hind*III**	**M**	M	M	*Eco*RI	M													
***Kpn*I**	**U**	seq	M	seq	M	*Kpn*I/M												
***Nco*I**	**H$^+$**	*Bam*HI	H	*Eco*RI	M	M	L											
***Nhe*I**	**A**	A	M	A	A	M	L	A										
***Not*I**	**U**	*Bam*HI	H	*Eco*RI	H	M	seq	SH	A									
***Pst*I**	**U**	*Bam*HI	H	*Eco*RI	M/H	M	seq	H	A	H								
***Pvu*II**	**M**	*Bam*HI	H	*Eco*RI	M	M	A	H	M	H	H							
***Sal*I**	**SH**	SH	SH	*Eco*RI	H	seq	seq	SH	seq	SH	SH	H						
***Sgr*BI**	**L**	L/M	M	M/H	M	M	L	M	L	seq	H	M	seq					
SlaI	**SH**	*Bam*HI	H	*Eco*RI	M	M	seq	SH	M	SH	H	H	SH	M/H				
***Sma*I**	**A**	A	seq	seq	A	A	A	A	A	A	A	A	seq	seq	seq			
***Sph*I**	**M**	M	M	*Eco*RI	M	M	L	M	L	*Not*I	M	M	SH	L	M	A		
***Sst*I**	**L**	L	M	seq	M	M	L	L	L	A	A	A	seq	L	seq	A	L	
***Xba*I**	**M**	M	M/H	*Eco*RI	M	M	L	M	A	*Not*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 perfomed 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.