Restriction Enzyme Activity in Promega 10X Buffers, Reaction Temperature and Heat Inactivation.

The 10X Reaction Buffer supplied with each restriction enzyme is optimized to give 100% activity. In many cases, good activity is also obtained using one of Promega's 4-CORE® 10X Buffers. Many commonly used cloning enzymes have buffers E and H as their optimal buffer, and so we have determined the activity of many of our enzymes in these buffers. This table may be used to select the best buffer for digestion with multiple restriction enzymes. Enzyme activity is expressed as a percent of the activity obtained with the optimal buffer for each enzyme.

Promega Enzyme	Buffer Supplied with Enzyme	Activity in							Heat Inactivation	Enzyme Assay Temperature
		A	B	C	D	E	H	MULTI-CORE™		
Aat II	J	50–75%	10–25%	<10%	<10%	10–25%	<10%	<10%	+	37°C
Acc I	G	50–75%	25–50%	25–50%	10–25%	<10%	<10%	25–50%	–	37°C
Acc III	F	<10%	10–25%	25–50%	25–50%	n.d.	n.d.	<10%	–	65°C
*Acc*65 I	D	10–25%	50–75%	75–100%	100%	75–100%	100–125%**	100%	+	37°C
*Acc*B7 I	E	10–25%	50–75%	100%*	<10%	100%	n.d.	100%	+	37°C
Age I	K	25–50%	25–50%	25–50%	50–75%	n.d.	n.d.	100%	+	37°C
Alu I	B	75–100%	100%	75–100%	10–25%	n.d.	n.d.	10–25%	+	37°C
*Alw*26 I	C	10–25%	25–50%	100%	10–25%	n.d.	n.d.	75–100%	+	37°C
*Alw*44 I	C	<10%	25–50%	100%	25–50%	n.d.	n.d.	100%	+	37°C
Apa I	A	100%	50–75%	50–75%	<10%	10–25%	<10%	75–100%	+	37°C
Ava I	B	10–25%	100%	50–75%	25–50%	100%	10–25%	<10%	+/–	37°C
Ava II	C	50–75%	50–75%	100%	25–50%	n.d.	n.d.	25–50%	+	37°C
Bal I	G	10–25%	<10%	<10%	<10%	n.d.	n.d.	<10%	+	37°C
*Bam*H I	E	75–100%*	75–100%	75–100%	50–75%	100%	50–75%	75–100%	+	37°C
Ban I	G	25–50%	25–50%	10–25%	<10%	n.d.	n.d.	100%	–	50°C
Ban II	E	75–100%	75–100%	75–100%	25–50%	n.d.	n.d.	100%	+	37°C
Bbu I	A	100%	75–100%	75–100%	<10%	10–25%	10–25%	100%	+	37°C
Bcl I	C	10–25%	75–100%	100%	50–75%	50–75%	50–75%	10–25%	–	50°C
Bgl I	D	10–25%	25–50%	75–100%	100%	25–50%	75–100%	100%	+	37°C
Bgl II	D	25–50%	75–100%	75–100%	100%	n.d.	n.d.	<10%	–	37°C
*Bsa*M I	D	10–25%	25–50%	50–75%	100%	n.d.	n.d.	25–50%	–	65°C
*Bsp*1286 I	A	100%	50–75%	25–50%	10–25%	n.d.	n.d.	75–100%	+	37°C
*Bsr*S I	D	10–25%	25–50%	10–25%	100%	n.d.	n.d.	100%	–	65°C
*Bss*H II	H	75–100%	50–75%	75–100%	50–75%	n.d.	100%	75–100%	–	50°C
*Bst*98 I	D	<10%	10–25%	10–25%	100%	n.d.	n.d.	25–50%	–	37°C
*Bst*E II	D	25–50%	50–75%	50–75%	100%	n.d.	n.d.	100%	–	60°C
*Bst*O I	C	10–25%	25–50%	100%	25–50%	n.d.	n.d.	<10%	–	60°C
*Bst*X I	D	<10%	10–25%	25–50%	100%	100%	75–100%	10–25%	+/–	50°C
*Bst*Z I	D	<10%	<10%	10–25%	100%	10–25%	75–100%	10–25%	–	50°C
*Bsu*36 I	E	<10%	25–50%	50–75%	25–50%	100%	n.d.	50–75%	–	37°C
Cfo I	B	75–100%	100%	75–100%	25–50%	n.d.	n.d.	100%	+/–	37°C
Cla I	C	75–100%	75–100%	100%	75–100%	100%	50–75%	100%	+	37°C
Csp I	K	<10%	10–25%	25–50%	50–75%	100%	100–125%**	10–25%	+	30°C
*Csp*45 I	B	25–50%	100%	50–75%	25–50%	100%	25–50%	50–75%	+	37°C
Dde I	D	25–50%	25–50%	50–75%	100%	n.d.	n.d.	25–50%	+/–	37°C
Dpn I	B	50–75%	100%	75–100%	50–75%	n.d.	n.d.	100%	+	37°C
Dra I	B	75–100%	100%	75–100%	50–75%	n.d.	n.d.	25–50%	+	37°C
*Ecl*HK I	E	<10%	<10%	75–100%	10–25%	100%	n.d.	50–75%	+	37°C
*Eco*47 III	D	<10%	25–50%	50–75%	100%	n.d.	n.d.	25–50%	+	37°C
*Eco*52 I	L	<10%	<10%	10–25%	25–50%	25–50%	50–75%	<10%	+	37°C
*Eco*ICR I	B	10–25%	100%	75–100%	<10%	25–50%	n.d.	100%	+	37°C
*Eco*R I	H	25–50%	50–75%	50–75%	50–75%	75–100%	100%	100%*	+	37°C
*Eco*R V	D	10–25%	25–50%	50–75%	100%	25–50%	50–75%	100%	+	37°C
Fok I	B	75–100%	100%	75–100%	25–50%	n.d.	n.d.	50–75%	+	37°C
Hae II	B	50–75%	100%	50–75%	10–25%	n.d.	n.d.	100%	–	37°C
Hae III	C	75–100%	75–100%	100%	50–75%	n.d.	n.d.	100%	–	37°C
Hha I	C	50–75%	75–100%	100%	50–75%	n.d.	n.d.	75–100%	+	37°C
*Hin*c II	B	25–50%	100%	25–50%	50–75%	75–100%	50–75%	100%	+	37°C
*Hin*d III	E	25–50%	100%	75–100%	10–25%	100%	25–50%	50–75%	+	37°C
*Hin*f I	B	50–75%	100%	75–100%	75–100%	n.d.	n.d.	50–75%	–	37°C
Hpa I	J	25–50%	50–75%	25–50%	10–25%	n.d.	n.d.	100%	–	37°C
Hpa II	A	100%	50–75%	50–75%	10–25%	n.d.	n.d.	100%	–	37°C

Restriction Enzyme Activity in Promega 10X Buffers, Reaction Temperature and Heat Inactivation (continued).

Promega Enzyme	Buffer Supplied with Enzyme	Activity in A	B	C	D	E	H	MULTI-CORE™	Heat Inactivation	Enzyme Assay Temperature
*Hsp*92 I	F	10–25%	75–100%	50–75%	25–50%	n.d.	n.d.	10–25%	+	37°C
*Hsp*92 II	K	10–25%	25–50%	25–50%	<10%	n.d.	n.d.	<10%	+	37°C
I-*Ppo* I	NA	10–25%	25–50%	25–50%	25–50%	n.d.	n.d.	—	+	37°C
Kpn I	J	100%*	25–50%	25–50%	<10%	25–50%	<10%	75–100%	+/–	37°C
Mbo I	C	10–25%	75–100%	100%	50–75%	n.d.	n.d.	<10%	+	37°C
Mbo II	B	10–25%	100%	50–75%	75–100%	n.d.	n.d.	100%	+	37°C
Mlu I	D	10–25%	25–50%	50–75%	100%	25–50%	100–125%**	10–25%	+/–	37°C
Msp I	B	75–100%	100%	75–100%	25–50%	n.d.	n.d.	25–50%	+	37°C
*Msp*A1 I	C	25–50%	100%*	100%	10–25%	n.d.	n.d.	100%	+	37°C
Nae I	A	100%	50–75%	25–50%	<10%	n.d.	n.d.	50–75%	+	37°C
Nar I	G	75–100%	50–75%	75–100%	25–50%	n.d.	n.d.	50–75%	+	37°C
Nci I	B	100%*	100%	25–50%	25–50%	n.d.	n.d.	50–75%	+	37°C
Nco I	D	50–75%	75–100%	75–100%	100%	100%	100–125%**	75–100%	+	37°C
Nde I	D	<10%	<10%	25–50%	100%	n.d.	n.d.	25–50%	+	37°C
Nde II	D	<10%	<10%	10–25%	100%	n.d.	n.d.	25–50%	+	37°C
*Ngo*M IV	MULTI-CORE™	100%*	100%*	100%*	<10%	n.d.	n.d.	100%	+	37°C
Nhe I	B	75–100%	100%	75–100%	10–25%	75–100%	10–25%	100%	+	37°C
Not I	D	<10%	10–25%	25–50%	100%	25–50%	100–125%**	25–50%	+	37°C
Nru I	K	<10%	<10%	<10%	50–75%	n.d.	n.d.	10–25%	+	37°C
Nsi I	D	10–25%	50–75%	50–75%	100%	25–50%	>125%**	10–25%	+/–	37°C
Pst I	H	10–25%	50–75%	50–75%	50–75%	25–50%	100%	25–50%	+	37°C
Pvu I	D	10–25%	25–50%	50–75%	100%	n.d.	n.d.	<10%	–	37°C
Pvu II	B	25–50%	100%	50–75%	25–50%	n.d.	n.d.	50–75%	+	37°C
Rsa I	C	75–100%	75–100%	100%	<10%	n.d.	n.d.	<10%	+	37°C
Sac I	J	75–100%	25–50%	25–50%	<10%	100%	25–50%	100%	+	37°C
Sac II	C	100%	50–75%	100%	50–75%	25–50%	>125%**	<10%	+	37°C
Sal I	D	<10%	10–25%	25–50%	100%	25–50%	25–50%	<10%	+	37°C
*Sau*3A I	B	25–50%	100%	75–100%	<10%	n.d.	n.d.	100%	+	37°C
*Sau*96 I	C	25–50%	25–50%	100%	50–75%	n.d.	n.d.	50–75%	–	37°C
Sca I	K	<10%	100%*	50–75%	75–100%	n.d.	n.d.	10–25%	+	37°C
Sfi I	B	75–100%	100%	75–100%	25–50%	75–100%	50–75%	75–100%	–	50°C
Sgf I	C	25–50%	25–50%	100%	<10%	n.d.	n.d.	<10%	+/–	37°C
Sin I	A	100%	75–100%	50–75%	10–25%	n.d.	n.d.	100%	+	37°C
Sma I	J	<10%	<10%	<10%	<10%	<10%	<10%	100%	+	25°C
*Sna*B I	B	50–75%	100%	50–75%	<10%	n.d.	n.d.	100%	–	37°C
Spe I	B	75–100%	100%	75–100%	75–100%	100%	25–50%	100%	+	37°C
Sph I	K	75–100%	75–100%	100%*	75–100%	100%	>125%**	10–25%	+	37°C
Ssp I	E	10–25%	50–75%	50–75%	75–100%	100%	100–125%**	50–75%	+	37°C
Stu I	B	75–100%	100%	75–100%	50–75%	n.d.	n.d.	50–75%	+	37°C
Sty I	F	25–50%	75–100%	75–100%	75–100%	10–25%	50–75%	<10%	+	37°C
Taq I	E	10–25%	25–50%	50–75%	50–75%	100%	n.d.	100%	–	65°C
*Tru*9 I	F	75–100%	50–75%	75–100%	25–50%	n.d.	n.d.	25–50%	–	65°C
*Tth*111 I	B	50–75%	100%	75–100%	25–50%	n.d.	n.d.	100%	–	65°C
Vsp I	D	<10%	25–50%	75–100%	100%	n.d.	n.d.	<10%	+	37°C
Xba I	D	50–75%	75–100%	75–100%	100%	100%	100–125%**	100%	–	37°C
Xho I	D	25–50%	75–100%	75–100%	100%	25–50%	100–125%**	10–25%	+	37°C
Xho II	C	25–50%	25–50%	100%	10–25%	n.d.	n.d.	<10%	+	37°C
Xma I	B	50–75%	100%	25–50%	<10%	25–50%	<10%	50–75%	+	37°C
Xmn I	B	75–100%	100%	75–100%	10–25%	n.d.	n.d.	75–100%	+	37°C

* Not recommended due to potential star activity.
** Unit activity is based on recommended buffer. In Buffer H, some enzymes have enhanced activity.
n.d. = Not determined.

Heat Inactivation Key:

+ = greater than 95% inactivation (DNA is undigested)
– = less than 95% inactivation (DNA digest is complete, i.e., ≥5% of the initial 20 activity units [≥1 unit] remains)
+/– = partial inactivation (DNA is partially digested)

Heat Inactivation Conditions:
Twenty units of enzyme in 50µl of its optimal buffer were heated at 65°C for 15 minutes.
One microgram of DNA is added and incubated for 1 hour in accordance with the unit definition, then analyzed by agarose gel electrophoresis.