

## Residual Enzyme Activity Following Inactivation Treatment

Heat treatment is generally applied to inactivate restriction enzymes after completion of a reaction. Heat tolerance varies depending on the kind of enzyme; however, in some cases heat treatment is insufficient to inactivate restriction enzymes completely. Therefore, four kinds of inactivation treatment were used for each enzyme, and the residual enzyme activity was measured to determine the best conditions for complete inactivation.

### Restriction Enzyme Properties

Restriction Enzyme	60°C 15 min.	70°C 15 min.	Ethanol Precipitation	Phenol Extraction
<i>Aat</i> II	+	-	-	NT
<i>Acc</i> I	+	-	-	NT
<i>Acc</i> II	+	+	-	NT
<i>Acc</i> III	NT	-	-	NT
<i>Afa</i> I	+	-	+	-
<i>Afl</i> II	-	NT	-	NT
<i>Alu</i> I	-	NT	+	-
<i>Aor</i> 13H I	NT	+	+	-
<i>Aor</i> 51H I	-	NT	-	NT
<i>Apa</i> I	-	NT	-	NT
<i>Apa</i> I	+	-	-	NT
<i>Ava</i> II	+	-	+	-
<i>Avi</i> II	+	-	+	-
<i>Bal</i> I	-	NT	+	-
<i>Bam</i> HI	-	NT	-	NT
<i>Ban</i> II	-	NT	-	NT
<i>Bcn</i> I	+	+	-	NT
<i>Bgl</i> I	-	NT	+	-
<i>Bgl</i> II	+	-	+	-
<i>Bln</i> I	+	+	-	NT
<i>Bpu</i> 1102 I	+	+	+	-
<i>Bsp</i> 1286 I	-	NT	-	NT
<i>Bsp</i> 1407 I	+	-	+	-
<i>Bsp</i> 1107 I	-	NT	-	NT
<i>Bss</i> HI	+	-	-	NT
<i>Bst</i> P I	NT	+	-	NT
<i>Bst</i> X I	-	NT	-	NT
<i>Bst</i> 1107 I	+	+	+	-
<i>Cfr</i> 10 I	+	+	+	-
<i>Cfr</i> 13 I	-	NT	-	NT
<i>Cla</i> I	-	NT	-	NT
<i>Cpo</i> I	-	NT	-	NT
<i>Dra</i> I	-	NT	-	NT
<i>Eae</i> I	-	NT	-	NT
<i>Eam</i> 1105 I	-	NT	+	-
<i>Eco</i> 0109 I	-	NT	-	NT
<i>Eco</i> 065 I	+	-	+	-
<i>Eco</i> R I	-	NT	-	NT
<i>Eco</i> R V	+	-	+	-
<i>Eco</i> T14 I	-	NT	-	NT
<i>Eco</i> T22 I	-	NT	-	NT
<i>Eco</i> S2 I	-	NT	+	-
<i>Eco</i> 81 I	+	-	-	NT
<i>Fba</i> I	+	+	-	NT
<i>Fok</i> I	-	NT	+	-
<i>Fse</i> I	-	NT	-	NT
<i>Hae</i> II	-	NT	-	NT
<i>Hae</i> III	+	-	-	NT
<i>Hap</i> II	+	-	+	-

Restriction Enzyme	60°C 15 min.	70°C 15 min.	Ethanol Precipitation	Phenol Extraction
<i>Hha</i> I	+	-	-	NT
<i>Hinc</i> II	+	-	-	NT
<i>Hind</i> III	+	-	-	NT
<i>Hinf</i> I	+	-	-	NT
<i>Hin</i> 1 I	-	NT	+	-
<i>Hpa</i> I	-	NT	-	NT
<i>Kpn</i> I	-	NT	-	NT
<i>Mbo</i> I	+	-	-	NT
<i>Mbo</i> II	+	-	-	NT
<i>Mfl</i> I	+	+	+	-
<i>Mlu</i> I	+	+	-	NT
<i>Msp</i> I	-	NT	+	-
<i>Mun</i> I	+	-	+	-
<i>Mva</i> I	-	NT	+	-
<i>Nae</i> I	-	NT	-	NT
<i>Nco</i> I	+	-	+	-
<i>Nde</i> I	+	-	-	NT
<i>Nhe</i> I	+	-	-	NT
<i>Not</i> I	+	+	+	-
<i>Nru</i> I	-	NT	+	-
<i>Pma</i> C I	-	NT	-	NT
<i>Psh</i> A I	-	NT	-	NT
<i>Psh</i> B I	+	-	-	NT
<i>Psp</i> 1406 I	-	NT	+	-
<i>Pst</i> I	-	NT	-	NT
<i>Pvu</i> I	-	NT	-	NT
<i>Pvu</i> II	+	+	+	-
<i>Sac</i> I	-	NT	-	NT
<i>Sac</i> II	+	-	+	-
<i>Sal</i> I	-	NT	-	NT
<i>Sau</i> 3A I	+	-	-	NT
<i>Sca</i> I	-	NT	-	NT
<i>Sfi</i> I	+	-	+	-
<i>Sma</i> I	-	NT	+	-
<i>Sna</i> B I	-	NT	-	NT-
<i>Spe</i> I	-	NT	-	NT
<i>Sph</i> I	-	NT	-	NT
<i>Sse</i> 8387 I	-	NT	-	NT
<i>Ssp</i> I	-	NT	-	NT
<i>Stu</i> I	-	NT	+	-
<i>Tth</i> HB8 I ( <i>Taq</i> I)	NT	+	+	-
<i>Tth</i> 111 I	NT	+	-	NT
<i>Van</i> 91 I	-	NT	+	-
<i>Vpa</i> K11B I	-	NT	-	NT
<i>Xba</i> I	-	NT	+	-
<i>Xho</i> I	+	+	-	NT
<i>Xsp</i> I	+	+	+	-

+: Residual activity remaining  
-: No residual activity  
NT: Not tested