

CUT-PROOF Restriction Enzymes

Aat II	$\frac{GACGTC}{CTGCAG}$	EBR-1001	250 unit	80,000
Acc III	$\frac{TCCGGA}{AGGCCIT}$	EBR-1005	250 unit	80,000
Alu I	$\frac{AGCT}{TCGA}$	EBR-1011	500 unit	80,000
ApaL I	$\frac{GTGCAC}{CACGTG}$	EBR-1015	1,000 unit	70,000
Ase I	$\frac{ATTAAT}{TAATTA}$	EBR-1017	2,500 unit	80,000
Ava II	$\frac{G GTCC}{CC TGG}$ A	EBR-1021	1,000 unit	80,000
BamH I	$\frac{G GATCC}{CCTAGG}$	EBR-1025	10,000 unit	50,000
Bbv I	$\frac{GCAGC(N)_8}{CGTCG(N)_{12}}$	EBR-1031	200 unit	70,000
Bgl I	$\frac{GCCN NN NGGC}{CGN NN NCCG}$	EBR-1035	1,000 unit	60,000
Dde I	$\frac{CTNAG}{GAN TC}$	EBR-1043	250 unit	70,000
Dpn II	$\frac{ GATC}{CTAG }$	EBR-1047	1,000 unit	70,000
Dra III	$\frac{CAC NN GTG}{GTG NN CAC}$	EBR-1051	2,500 unit	70,000
EcoR I	$\frac{G AATTC}{CTTAA G}$	EBR-1055	10,000 unit	50,000
Fok I	$\frac{GGATG(N)_9}{CCTAC(N)_{13}}$	EBR-1059	1,000 unit	80,000
Hae III	$\frac{GG CC}{CC GG}$	EBR-1065	2,500 unit	70,000
Hinc II	$\frac{GTPy PuAC}{CAPu PyTG}$	EBR-1071	1,000 unit	70,000
Hinf I	$\frac{G ANTC}{CTNA G}$	EBR-1075	2,500 unit	70,000
Hpa II	$\frac{C CGG}{GG CC}$	EBR-1079	1,000 unit	70,000
Kpn I	$\frac{GGTAC C}{C CATGG}$	EBR-1083	5,000 unit	70,000
Mbo II	$\frac{GAAGA(N)_8}{CTTCT(N)_7}$	EBR-1087	250 unit	80,000
Mwo I	$\frac{GC NNNN NNGC}{CG NN NNNN CG}$	EBR-1091	250 unit	80,000
Nco I	$\frac{C CATGG}{GGTACC}$	EBR-1097	1,000 unit	60,000

Acc I	$\frac{AT}{GT CGAC}$ CA TA TG GC	EBR-1003	250 unit	80,000
Afl II	$\frac{C TTAAG}{GAA TT C}$	EBR-1201	2,500 unit	80,000
Apa I	$\frac{GGGCC C}{C CCGGG}$	EBR-1013	2,500 unit	60,000
Apo I	$\frac{Pu AATTPy}{Py TTA APu}$	EBR-1203	500 unit	80,000
Ava I	$\frac{C PyCGPuG}{G PuGC Py C}$	EBR-1019	2,500 unit	80,000
Ava III	$\frac{ATGCA T}{T ACGTA}$	EBR-1023	1,000 unit	80,000
Ban I	$\frac{GG PyPuCC}{CC PuPy GG}$	EBR-1027	2,500 unit	70,000
Bcl I	$\frac{T GATCA}{A CTAG T}$	EBR-1033	1,000 unit	60,000
Bgl II	$\frac{A GATCT}{T CTAG A}$	EBR-1037	2,500 unit	60,000
Dpn I	$\frac{CH_3}{G ATC}$ CT AG CH ₃	EBR-1045	500 unit	70,000
Dra I	$\frac{TTT AAA}{AAA TTT}$	EBR-1049	1,000 unit	70,000
Eag I	$\frac{C GGCCG}{G CCG GC}$	EBR-1053	500 unit	70,000
EcoR V	$\frac{GAT ATC}{CTA TAG}$	EBR-1057	5,000 unit	70,000
Hae II	$\frac{PuGCGC Py}{Py C CGPu}$	EBR-1063	1,000 unit	70,000
Hae IV	$\frac{(N)_7 GAPyNNNNPuTC(N)_{14}}{(N)_{13} CTPuNNNNPyAG(N)_9}$	EBR-1067	500 unit	80,000
Hind III	$\frac{A AGCTT}{TTCG AA}$	EBR-1073	10,000 unit	60,000
Hpa I	$\frac{GTT AAC}{CAA TTG}$	EBR-1077	500 unit	70,000
Kas I	$\frac{G GCGCC}{CCG CGG}$	EBR-1205	500 unit	70,000
Mbo I	$\frac{ GATC}{CTAG }$	EBR-1085	500 unit	80,000
Mlu I	$\frac{A CGCGT}{TGGC AA}$	EBR-1089	1,000 unit	80,000
Nae I	$\frac{GCC GGC}{CGG CCG}$	EBR-1093	500 unit	80,000
Nde I	$\frac{CA TATG}{GTAT AC}$	EBR-1099	5,000 unit	70,000

Nhe I	$\frac{G C T A G C}{C G A T C G}$	EBR-1101	1,000 unit	70,000
Pho I	$\frac{G G C C}{C C G G}$	EBR-1107	500 unit	80,000
Pvu II	$\frac{C A G C T G}{G T C G A C}$	EBR-1113	2,500 unit	70,000
Sac II	$\frac{C C G C G G}{G G C G C C}$	EBR-1117	1,000 unit	70,000
Sca I	$\frac{A G T A C T}{T C A T G A}$	EBR-1123	1,000 unit	70,000
Sma I	$\frac{C C C G G G}{G G G C C C}$	EBR-1127	2,500 unit	70,000
Sph I	$\frac{G C A T G C}{C G T A C G}$	EBR-1133	500 unit	80,000
TthHB8 I	$\frac{T C G A}{A G C T}$	EBR-1145	2,500 unit	70,000
Xho I	$\frac{C T C G A G}{G A G C T C}$	EBR-1149	5,000 unit	60,000

Not I	$\frac{G C G G C C G C}{C G C C G G C G}$	EBR-1103	500 unit	70,000
Pst I	$\frac{C T G C A G}{G A C G T C}$	EBR-1109	10,000 unit	60,000
Sac I	$\frac{G A G C T C}{C I T C G A G}$	EBR-1115	2,500 unit	70,000
Sal I	$\frac{G T C G A C}{C A G C T G}$	EBR-1119	2,500 unit	70,000
Sfi I	$\frac{G G C C N N N N N G G C C}{C C G G N N N N N C C G G}$	EBR-1125	2,500 unit	80,000
Spe I	$\frac{A C T A G T}{T G A T C A}$	EBR-1131	500 unit	70,000
Ssp I	$\frac{A A T A T T}{T T A T A A}$	EBR-1135	500 unit	70,000
Xba I	$\frac{T C T A G A}{A G A T C T}$	EBR-1147	5,000 unit	70,000
Xma I	$\frac{C C C G G G}{G G G C C C}$	EBR-1153	250 unit	70,000

Pu = A or G Py = C or T N = A or C or G or T



Buffer Composition (1x)

Buffer 1 : 10 mM Bis Tris Propane-HCl, pH7.0, 10 mM MgCl₂, 1 mM DTT

Buffer 2 : 10 mM Tris-HCl, pH7.9, 50 mM NaCl, 10 mM MgCl₂, 1 mM DTT

Buffer 3 : 50 mM Tris-HCl, pH7.9, 100 mM NaCl, 10 mM MgCl₂, 1 mM DTT

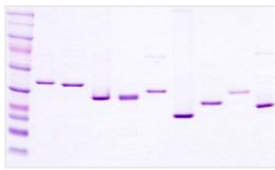
Buffer 4 : 20 mM Tris-acetate, pH7.9, 10 mM magnesium acetate, 50 mM potassium acetate, 1 mM DTT

Buffer BamH I : 10 mM Tris-HCl, pH7.9, 150 mM NaCl, 10 mM MgCl₂, 1 mM DTT

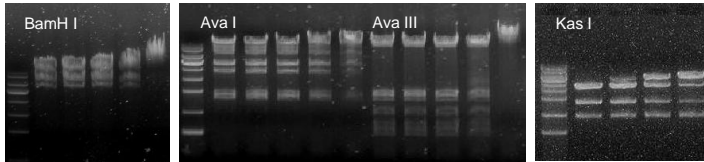
Buffer EcoR I : 100 mM Tris-HCl, pH7.5, 50 mM NaCl, 10 mM MgCl₂, 0.025% Triton X-100

Quality Control

- Purity by SDS-PAGE:** Purified restriction endonucleases are analyzed on a SDS-PAGE.

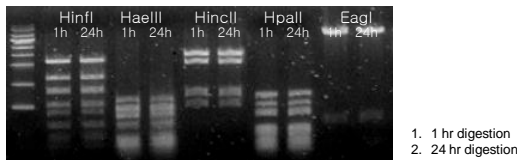


- Unit Determination:** One unit of restriction endonuclease activity is defined as the amount of enzyme required to completely digest 1 µg of substrate DNA (lambda DNA or plasmid DNA) in a total reaction volume of 10 µl in one hour using the buffer provided. Incubations are performed in capped microcentrifuge tubes at the appropriate incubation temperature. Concentrated enzymes are diluted to approximately 1,000 units/ml using the recommended storage buffers before determining their activity.

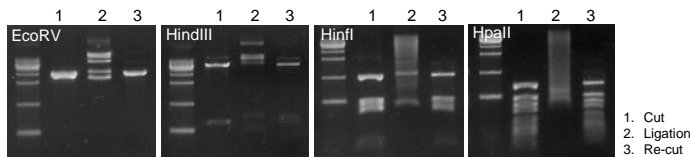


- Assay for Non-specific Endonucleases:** To assay for non-specific endonuclease contamination, each restriction endonuclease is incubated with a supercoiled plasmid substrate lacking the recognition sequence of the restriction endonuclease. A single non-specific nick in the RF I DNA converts it to the RF II form (nicked circle). Aliquots are incubated with 1 µg of RF I (supercoiled form) DNA in a reaction volume of 10 µl using the recommended reaction buffer. Incubations are performed for 4 hours at the recommended temperature. The two forms are easily distinguished on agarose gels and the percent conversion from RF I to RF II is determined.

- Assay for Nuclease Contamination:** All restriction endonucleases are incubated overnight in their recommended reaction buffer with 1 µg of substrate DNA in a volume of 10 µl. The characteristic banding pattern produced by the enzyme for one hour is compared to the pattern produced from an excess of enzyme incubated overnight. A sharp, unaltered pattern under these conditions is an indication that the enzyme preparation is free of detectable levels of non-specific DNases. The maximum number of units that can be incubated overnight is reported.



- Ligation of DNA Fragments:** DNA fragments are produced by an excessive over-digestion of substrate DNA with each restriction endonuclease. These fragments are then ligated with T4 DNA Ligase at a 5' termini concentration of 0.1–1.0 µM. The ligated fragments are then recut with the same restriction endonuclease. Ligation can only occur if the 3' and 5' termini are left intact, and only those molecules with a perfectly restored recognition site can be recleaved. A normal banding pattern after cleavage indicates that both the 3' and 5' termini are intact and the enzyme preparation is free of detectable exonucleases and phosphatases.



- Blue/White Screening Assay:** Enzymes used for cloning applications are tested by an additional quality control, the Blue/White Screening Assay, to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. The assay is performed by digesting an appropriate vector at a unique site within the lacZ α gene with a 10-fold excess of enzyme, ligating, transforming and plating on X-Gal/IPTG/Amp plates. Successful cleavage, ligation and expression of β -galactosidase is a function of how intact its gene remains after cloning. An intact gene gives rise to a blue colony. While an interrupted gene (i.e., degraded DNA end) gives rise to a white colony. All restriction enzymes tested must produce fewer than 1% white colonies in order to be Blue/White certified.