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Restriction Endonuclease *Eco* RI

From *Escherichia coli* BS5

Cat. No. 10 703 737 001	5,000	units (10 U/µl)
Cat. No. 11 175 084 001	10,000	units (10 U/µl)
Cat. No. 10 200 310 001	10,000	units, high concentration (40 U/µl)
Cat. No. 10 606 189 001	50,000	units, high concentration (40 U/µl)

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Store at −15 to −25°C

Stability/Storage The undiluted enzyme solution is stable when stored at −15 to −25°C until the control date printed on the label. Do not store below -25°C to avoid freezing.
Note: Product is shipped on dry ice.

Sequence specificity *Eco* RI recognizes the sequence G/AATTC and generates fragments with 5´-cohesive termini (1).

Compatible ends *Eco* RI generates compatible ends to *Acs* I and *Mun* I.

Enzyme with compatible ends	Recognition sequence	New sequence if *Eco* RI is ligated to enzyme with compatible ends		Enzyme that can cut this new sequence
		Eco RI - Enzyme	Enzyme – *Eco* RI	
Acs I	(A,G)/AATT(C,T)	G/AATT(C,T)	(A,G)/AATTC	*Acs* I, *Eco* RI
***Eco* RI**	**G/AATTC**	**G/AATTC**	**G/AATTC**	***Eco* RI+ *Rsr* I**
Mun I	C/AATTG	G/AATTG	C/AATTC	*Tsp* EI

Isoschizomers *Eco* RI is an isoschizomer to *Rsr* I.

Methylation sensitivity *Eco* RI is inhibited by the presence of N^6-methyladenine at either or both A residues, and by the the presence of 5-methylcytosine as indicated (*).

Storage buffer 10 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 0.5 mM Dithioerythritol, 0.2% Triton X-100 (v/v), 50% Glycerol (v/v), pH approx. 7.2 (at 4°C).

Incubation buffer (10x, included) SuRE/Cut Buffer **H**: 0.5 M Tris-HCl, 1 M NaCl, 100 mM $MgCl_2$, 10 mM DTE, pH 7.5 (at 37°C).

Activity in SuRE/Cut Buffer System Bold face printed buffer indicates the recommended buffer for optimal activity:

A	B	L	M	**H**
100%	100%	25-50%	50-75%	**100%**

Incubation temp. **37°C**

Unit definition One unit is the enzyme activity that completely cleaves 1µg λDNA in 1 h at **37°C** in a total volume of 50 µl in SuRE/Cut **Buffer H.** 1 µg pBR322 DNA is digested completely by approx. 2 units of *Eco* RI because of the larger number of cleavage sites per µg pBR322 DNA as compared to λDNA.

Typical experiment

Component	**Final concentration**
DNA	1 µg
10× SuRE/Cut Buffer **H**	5 µl
Repurified water	Up to a total volume of 50 µl
Restriction enzyme	1 unit

Incubate at **37°C** for 1 h.

Heat inactivation *Eco* RI can be heat inactivated by 15 min incubation at 65°C (tested up to 10 U/µg DNA).

Number of cleavage sites on different DNAs (2):

λ	Ad2	SV40	Φ X174	M13mp7	pBR322	pBR328	pUC18
5	5	1	0	2	1	1	1

PFGE tested *Eco* RI has been tested in Pulsed Field Gel Electrophoresis (test system bacterial chromosomes). For cleavage of genomic DNA (*E. coli* C 600) embedded in agarose for PFGE analysis, 10 units of enzyme/µg DNA and 4 h incubation time are recommended.

Activity in PCR buffer Relative activity in PCR mix (Taq DNA Polymerase buffer) is 50%. The PCR mix contained λ target DNA, primers, 10 mM Tris-HCl (pH 8.3, 20°C), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTPs, 2.5 U Taq DNA polymerase. The mix was subjected to 25 amplification cycles.

Ligation and recutting assay *Eco* RI fragments obtained by complete digestion of 1 µg λDNA are ligated with 1 U T4 DNA Ligase (Cat. No. 10 481 220 001) in a volume of 10 µl by incubation for 16 h at 4 °C in 66 mM Tris-HCl, 5 mM $MgCl_2$, 5 mM Dithioerythritol, 1 mM ATP, pH 7.5 (at 20°C) resulting in >95% recovery of 1 µg λDNA × *Eco R*I fragments. Subsequent re-cutting with *Eco* RI yields > 95% of the typical pattern of λDNA × *Eco R*I fragments.

Troubleshooting A critical component is the DNA substrate.
Many compounds used in the isolation of DNA, for example, phenol, chloroform, EtOH, SDS, high levels of NaCl, metals (*e.g.*, Hg^{2+}, Mn^{2+}), inhibit or alter recognition specificity of many restriction enzymes.
Such compounds should be removed by EtOH precipitation followed by drying, before the DNA is added to the restriction digest reaction.
Appropriate mixing of the enzyme is recommended.

Quality control Lot-specific certificates of analysis are available at www.lifescience.roche.com/certificates.

Absence of unspecific endonuclease activities 1 µg λDNA is incubated for 16 h in 50 µl SuRE/Cut Buffer H with excess of *Eco* RI. The number of enzyme units which do not change the enzyme-specific pattern is stated in the certificate of analysis.

Absence of exonuclease activity Approx. 5 µg [^{3}H] labeled calf thymus DNA are incubated with 3 µl *Eco* RI for 4 h at 37°C in a total volume of 100 µl 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM Dithioerythritol, pH approx. 7.5. Under these conditions, no release of radioactivity is detectable, as stated in the certificate of analysis.

References

1 Hedgpeth, J. *et al.* (1972) *Proc. Natl. Acad. Sci USA* **69**, 3448.
2 Polisky, B. *et al.* (1975) *Proc. Natl. Acad. Sci USA* **72**, 3310.
3 Tikchonenko, T. I. *et al.* (1978) *Gene* **4**, 195.
4 Kessler, C. & Manta, V. (1990) *Gene* **92**, 1–250.
5 Rebase The Restriction Enzyme Database: http://rebase.neb.com
6 Benchmate: http://biochem.roche.com/benchmate
7 Alves, J. *et al.* (1984) "The influence of sequence adjacent to the recognition site on the cleavage of oligonucleotides by the Eco RI endonuclease" *Eur. J. Biochem.* 140, 83-92.
8 Bischofberger, N. *et al.* (1987) "Cleavage of single stranded oligonucleotides by Eco RI restriction endonuclease" *Nucleic Acids Res.* 15, 709-716.
9 Chen, J. *et al.* (1990) *Nucleic Acids Res.* 18, 3255-3260.
10 Halford, S.E. *et al.* (1980) *Biochem. J.* 191, 581-592.
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12 Mayer, H. (1978) "Optimization of the Eco RI*-activity of Eco RI endonuclease" *FEBS Lett.* 90 (2): 341-344.
13 Rosenberg, J.M. *et al.* (1987) "Structure and recognition mechanism of Eco RI endonuclease" *TIBS* 12, 395-398.
14 Woodhead, D.L. & Malcolm, A.D.B. (1981) *Eur. J. Biochem.* 120, 125-128.

Ordering Information

Product	Application	Pack Size	Cat. No.
Restriction Enzymes	DNA restriction digestion.	Please refer to website	
T4 DNA Ligase	Ligation of sticky- and blunt-ended DNA fragments.	100 U 500 units (1 U/µl) 500 units (5 U/µl)	10 481 220 001 10 716 359 001 10 799 009 001
SuRE/Cut Buffer Set for Restriction Enzymes	Incubation buffers A, B, L, M and H for restriction enzymes.	1 ml each (10× conc. solutions)	11 082 035 001
SuRE/Cut Buffer A	Restriction enzyme incubation.	5 × 1 ml (10× conc. solution)	11 417 959 001
SuRE/Cut Buffer B	Restriction enzyme incubation.	5 × 1 ml (10× conc. solution)	11 417 967 001
SuRE/Cut Buffer H	Restriction enzyme incubation.	5 × 1 ml (10× conc. solution)	11 417 991 001
SuRE/Cut Buffer L	Restriction enzyme incubation.	5 × 1 ml (10× conc. solution)	11 417 975 001
SuRE/Cut Buffer M	Restriction enzyme incubation.	5 × 1 ml (10× conc. solution)	11 417 983 001
Water, PCR Grade	Specially purified, double-distilled, deionized, and autoclaved.	100 ml (4 vials of 25 ml) 25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml)	03 315 843 001 03 315 932 001 03 315 959 001

Changes to previous version

Editorial changes

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Commonly used bacterial strains

Strain	Genotype
BL21	*E. coli B F⁻ dcm ompT hsdS(r_B- m_B-) gal* (Studier, F.W. *et al* (1986) *J. Mol. Biol.*, **189,** 113.)
C600[e]	*supE44 hsdR2 thi-1 thr-1 leuB6 lacY1 tonA21*; (Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557.)
DH5α	*supE44* Δ(*lac*U169 (φ80d*lacZ*ΔM15) *hsd*R17 *rec*A1 *end*A1 *gyr*A96 *thi*-1 *rel*A1; (Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557.)
HB101	*supE44 hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*; (Hanahan, D., (1983) *J. Mol. Biol.* **166**, 557.)
JM108	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ(*lac-pro*AB); (Yanisch- Perron, C. *et al.,* (1985) *Gene* **33**, 103.)
JM109	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ(*lac-pro*AB) F'[*traD36proAB*$^+$, *lacI*^{q} *lacZ*ΔM15]; (Yanisch- Perron, C. *et al.,* (1985) *Gene* **33**, 103.)
JM110	*rpsL (Str^r) thr leu thi-l lacY galK galT ara tonA tsx dam dcm supE44 Δ(lac-proAB) F'[traD36proAB$^+$, lacI^q lacZΔM15];* (Yanisch- Perron, C. *et al.,* (1985) *Gene* **33**, 103.)
K802	*supE hsdR gal metB;* (Raleigh, E. et al., (1986) Proc.Natl. Acad.Sci USA, 83, 9070.; Wood, W.B. (1966) *J. Mol. Biol.*, **16**, 118.)
SURE^r	*recB recJ sbc C201 uvrC umuC::Tn5(kan^r) lac ,* Δ(hsdRMS) endA1 gyrA96 thi relA1 supE44 F'[proAB$^+$ lacI^q lacZΔM15 Tn10 (tet^r); (Greener, A. (1990) *Stratagies*, **3**, 5.)
TG1	*supE hsd Δ5 thi* Δ(*lac-pro*AB) F'[*traD36proAB*$^+$, *lacI*^{q} *lacZ*ΔM15]; (Gibson, T.J. (1984) PhD Theses. Cambridge University, U.K.)
XL1-Blue^r	*supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac* F'[*proAB*$^+$, *lacI*^{q} *lacZ*ΔM15 Tn10 *(tet^r)*]; (Bullock *et al.,* (1987) *BioTechniques*, 5, 376.)

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