Print

For life science research only.
Not for use in diagnostic procedures.

Restriction Endonuclease *Eco* 47 III

From Escherichia coli RFL 47

Cat. No. 11 167 103 001 100 units (5 U/µl)

Version 16
Content version: July 2017
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.

Sequence specificity *Eco* 47 III recognizes the sequence AGC/GCT and generates fragments blunt ends (1).

Compatible ends The enzyme generates compatible ends to any blunt end.

Isoschizomers The enzyme is not known to have isoschizomers.

Methylation sensitivity *Eco* 47 III is inhibited by the presence of 5-methylcytosine as indicated (*). 6-metyladenine is not inhibiting, as indicated (°).

Storage buffer 10 mM Tris-HCl, 100 mM KCl, 0.1 mM EDTA, 10 mM dithiothreitol, 200 µg/ml bovine serum albumin, 50% glycerol (v/v), pH approx. 8.0 (at 4° C).

Incubation buffer, (10x) 500 mM Tris-HCl, 1 MNaCl, 100 mM $MgCl_2$, 10 mM Dithioerythritol, pH 7.5 (at 37° C), (= SuRE/Cut Buffer **H)**

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

A	B	L	M	**H**
25-50%	50-75%	0-10%	25-50%	**100%**

Incubation temperature **37°C**

Unit definition One unit is the enzyme activity that completely cleaves 1 µg λDNA in 1 h at **37° C** in the SuRE/Cut buffer **H** in a total volume of 25 µl. 1 µg pBR322 is digested completely by 4 units of *Eco* 47 III.

Typical experiment

Component	Final concentration
DNA	1 µg
10 × SuRE/Cut Buffer **H**	2.5 µl
Repurified water	Up to a total volume of 25 µl
Restriction enzyme	1 unit

Incubate at **37°C** for 1 h.

Heat inactivation The enzyme can be heat inactivated by 15 min incubation at 65° C (tested up to 100 units/µg DNA).

Number of cleavage sites on different DNAs (2):

λ	Ad2	SV40	Φ X174	M13mp7	pBR322	pBR328	pUC18
2	13	1	0	2	4	3	0

PFGE tested *Eco* 47 III has been tested in Pulsed-Field-Gel Electrophoresis (test system bacterial chromosomes). For cleavage of genomic DNA (*E.*coli C600) 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 **0%**. 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.

Troubleshooting A critical component is the DNA substrate. Many compounds used in the isolation of DNA such as phenol, chloroform, ethanol, SDS, high levels of NaCl, metal ions (*e.g.*, Hg^{2+}, Mn^{2+}) inhibit or alter recognition specificity of many restriction enzymes. Such compounds should be removed by ethanol 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* 47 III. 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* 47 III 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. The release of radioactivity is calculated as a percentage value of liberated to input radioactivity per unit of enzyme (stated in the certificate of analysis).

Ligation and recutting assay *Eco* 47 III fragments obtained by complete digestion of 1 µg pBR322 DNA 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 Dithiothreitol, 1 mM ATP, pH 7.5 (at 20° C).

The percentage of ligation and subsequent recutting with *Eco* 47 III which yields the typical pattern of pBR322 × *Eco* 47 III fragments are determined and stated in the certificate of analysis.

References

1 Janulaitis, A., Petrusyté, M. & Butkus, V. (1983) *FEBS Lett.* **161**, 213–216.
2 Kessler, C. & Manta, V. (1980) *Gene* **92**, 1–248.
3 Sagawa, H. et al. (1992) Nucl. Acids Res. 20, 365;
4 Rebase The Restriction Enzyme Database: http://rebase.neb.com

Ordering Information

Product	Application	Packsize	Cat. No.
T4 DNA Ligase	Ligation of sticky- and blunt- ended DNA fragments.	100 U 500 units (1 U/µl)	10 481 220 001 10 716 359 001
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

Trademarks

HIGH PURE and SURE/CUT are trademarks of Roche. All other product names and trademarks are the property of their respective owners.

Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

Disclaimer of License

For patent license limitations for individual products please refer to: List of biochemical reagent products

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*$^+$, *lac*I^q *lac*ZΔM15]; (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* Δ*5 thi* Δ(*lac-pro*AB) F'[*traD36proAB*$^+$, *lac*I^q *lac*ZΔM15]; (Gibson, T.J. (1984) PhD Theses. Cambridge University, U.K.)
XL1-Blue^r	*supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac* F'[*proAB*$^+$, *lac*I^q *lac*ZΔM15 Tn10 *(tet^r)*]; (Bullock *et al.,* (1987) BioTechniques, 5, 376.)

Contact and Support

To ask questions, solve problems, suggest enhancements and report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com** and select your home country. Country-specific contact information will be displayed.

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany