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Restriction Endonuclease Sal I

From *Streptomyces albus G*

Cat. No. 10 348 783 001 500 units (10 U/µl)
Cat. No. 10 567 663 001 2500 units (10 U/µl)
Cat. No. 11 047 612 001 2500 units , high concentration (40 U/µl)

Version 20
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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.

Sequence specificity *Sal* I recognizes the sequence G/TCGAC and generates fragments with 5´-cohesive termini (1).

Compatible ends *Sal* I generates compatible ends to *Xho* I.

Enzyme with compatible ends	Recognition sequence	New sequence if *Sal* I is ligated to enzyme with compatible ends		Enzyme that can cut this new sequence
		Sal I - Enzyme	Enzyme – *Sal* I	
***Sal* I**	**G/TCGAC**	**G/TCGAC**	**G/TCGAC**	***Sal* I**
***Xho* I**	C/TCGAG	G/TCGAG	C/TCGAC	*Taq* I

Isoschizomers The enzyme is not known to have commercially available isoschizomers.

Methylation sensitivity *Sal* I is inhibited by the presence of 5-methylcytosine at G/T^{m}CGAC and N^{6}-methyladenine at G/TCG^{m}AC.

Storage buffer 10 mM Tris-HCl, 1 mM EDTA, 10 mM Dithioerythritol, 0.05 % Polydocanol, 50% Glycerol (v/v), pH ca. 7.5 (at 4°C).

Suppl. Incubation buffer (10x) 500 mM Tris-HCl, 1 M NaCl, 100 mM $MgCl_2$, 10 mM Dithioerythrithol, 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**
0-10%	25-50%	0-10%	10-25%	**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 a total volume of 50 µl SuRE/Cut buffer **H**. 1 µg pBR322 DNA is digested completely by ca. 5 units of *Sal* I on account of the larger number of cleavage sites per µg of pBR322 DNA as compared to λDNA.

Typical experiment

Component	**Final concentration**
DNA	1 µg
10 × SuRE/Cut Buffer **H**	5.0 µl
Sterile redist. water	Up to a total volume of 50 µl
Restriction enzyme	1 unit

Incubate at **37°C** for 1 h.

Heat Inactivation *Sal* I can be heat-inactivated by 15 min incubation at 65°C (tested up to 100 U/µg DNA).

Number of cleavage sites on different DNAs (2):

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

PFGE tested *Sal* I has been tested in Pulsed-Field Gel Electro- phoresis (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 **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. After addition of 100 mM NaCl to the RE digest in the PCR mix, the activity of *Sal* I could be improved to 40%.

Star activity The most common star activity is the relaxed specificity, *i.e.* the enzyme not only recognizes the 6 bp palindrome but also the 4 bp core sequence. *Sal* I exhibits star activity in low salt (buffer **H** is recommended) and in high Glycerol; pH also seems to play a role in exhibition of star activity: pH 8.0 is already too high. Do not leave *Sal I* at room temperature, not even for a short period.

Ligation and recutting assay *Sal* I fragments obtained by complete digestion of 1 µg pBR322 DNA are ligated with 1 unit 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 pBR322 DNA.

Subsequent re-cutting with *Sal* I yields > 95% of the typical pattern of pBR322 × *Sal* I fragments.

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 *Sal* I. 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 *Sal* I 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 Arrand, J. R. et al. (1978) *J. Mol. Biol.* **118**, 127.
2 Kessler, C. & Manta, V. (1990) *Gene* **92**, 1–248.
3 Rebase The Restriction Enzyme Database: http://rebase.neb.com
4 Benchmate: http://www.roche-applied-science.com/benchmate

Ordering Information

Product	Application	Packsize	Cat. No.
Rapid DNA Ligation Kit	Ligation of sticky- or blunt-ended DNA fragments in just 5 min at 15 – 25 °C.	Kit (40 DNA ligations)	11 635 379 001
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
rAPid Phosphatase	Dephosphorylation of 5´-phosphate residues from nucleic acids	1000 U 5000 U	04 898 133 001 04 898 141 001
rAPid Dephos and Ligation Kit	Dephosphorylation of nucleic acids.	40 reactions 160 reactions	04 898 117 001 04 898 125 001
Alkaline Phosphatase (AP), special quality for molecular biology	Dephosphorylation of 5´-phosphate residues from nucleic acids.	1000 U (20 U/µl)	11 097 075 001
Agarose MP	Multipurpose agarose for analytical and preparative electrophoresis of nucleic acids	100 g 500 g	11 388 983 001 11 388 991 001
Agarose LE	Separation of nucleic acids in the range 0.2 – 1.5 kbp	100 g 500 g	11 685 660 001 11 685 678 001
Agarose Gel DNA Extraction Kit	For the elution of DNA fragments from agarose gels.	1 Kit (max. 100 reactions)	11 696 505 001
High Pure PCR Product Purification Kit	Purification of PCR or enzymatic modification reaction (*e.g.* restriction digest)	50 purifications 250 purifications	11 732 668 001 11 732 676 001
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
BSA, special quality for molecular biology	Maintaining enzyme stability	20 mg (1 ml)	10 711 454 001

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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