

# Restriction Endonuclease Ksp I (Sac II)

From Kluyvera species

**Cat. No. 11 117 807 001** 1000 U (10 U/μl)

CCGCGG GGCGCC

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Store at -15 to -25°C

Stability/Storage

The undiluted enzyme solution is stable when stored at -15 to  $-25^{\circ}$ C until the control date printed on the label. Do not store below  $-25^{\circ}$ C to avoid freezing. **Note:** Product is shipped on dry ice.

Sequence specificity

Ksp I recognizes the sequence CCGC/GG and generates fragments with 3'-cohesive ends (1).

Isoschizomers

Ksp I is an isoschizomer to Sac II and Sst II.

Methylation sensitivity

Ksp I is not sensitive to the presence of 5'-methylcytosine at the 5'-residue in contrast to its isoschizomer Sac II

Storage buffer

20 mM Tris-HCl, 100 mM NaCl, 0.1 mM EDTA, 10 mM 2-mercaptoethanol, 0.2% polydocanol (v/v), 0.01% gelatine, 50% glycerol (v/v), pH approx. 7.5 (at 4°C).

Incubation buffer (10x, included)

100 mM Tris-HCl, 100 mM MgCl $_2$ , 10 mM dithioerythritol, pH 7.5 (at 37°C) (= SuRE/Cut Buffer  $\bf L$ )

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

Α	В	L	M	Н
0-10%	0-10%	100%	0-10%	0-10%

Incubation temperature

37°C

Unit definition

One unit is the enzyme activity that completely cleaves 1  $\mu$ g  $\lambda$  DNA in 1 h at **37°C** in the SuRE/Cut Buffer **L** in a total volume of 25  $\mu$ l.

Typical experiment

Component	Final concentration
DNA	1 μg
10 × SuRE/Cut Buffer <b>L</b>	2.5 μl
Sterile redist. water	Up to a total volume of 25 μl
Restriction enzyme	1 U

Incubate at 37°C for 1 h.

**Heat inactivation** 

Ksp I cannot be inactivated by 15 min incubation at 65°C.

### Number of cleavage sites on different DNAs (2)

Ksp I belongs to the class of "rare-cutter" enzymes. The most rarely occurring trinucleotide sequences in pro-karyoric DNA are CCG and GGC. Ksp I cleaves bacterial genomic DNA to produce fragments 20-50 kb in size. Yeast genomic DNA (AT-rich) is cleaved to generate fragments in the range of 30 kb. The average fragment size produced by Ksp I from cleavage of mammalian genomic DNA is in the range of 100 kb. Ksp I has two slow cleavage sites on adenovirus DNA.

λ	Ad2	SV40	Φ X174	M13mp7	pBR322	pBR328	pUC18
4	33	0	1	0	0	0	0

Ligation and recutting assay

*Ksp* I fragments obtained by complete digestion of 1 μg λDNA are ligated with 1 U T4-DNA ligase (Cat. No. 481 220) in a volume of 10 μl by incubation for 16 h at 4°C in 66 mM Tris-HCl, 5 mM MgCl<sub>2</sub>, 5 mM dithiothreitol, 1 mM ATP, pH 7.5 (at 20°C) resulting in >90 % recovery of 1 μg λDNA × *Ksp* I fragments. Subsequent re-cutting with *Ksp* I yields > 90% of the typical pattern of λDNA × *Ksp* I fragments.

**PFGE** tested

Ksp I 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 the use of 10 U enzyme/μg DNA and 4 h incubation time at 37°C is recommended.

**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<sup>2+</sup>, Mn<sup>2+</sup>) 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

Absence of exonuclease activity 1  $\mu g~\lambda DNA$  is incubated for 16 h in 50  $\mu l$  SuRE/Cut Buffer L with excess of Ksp l. The number of enzyme units which do not change the enzyme-specific pattern is stated in the certificate of analysis.

Approx. 5  $\mu$ g [ $^3$ H] labeled calf thymus DNA are incubated with 3  $\mu$ l Kspl for 4 h at 37 $^\circ$ C in a total volume of 100  $\mu$ l 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

- Bolton, B. J., Schmitz, G., Jarsch, M. & Kessler, C. (1989) Nucleic Acids Res., 17, No. 22, 9476.
- Acids Res., **17**, No. 22, 9476. 2 Kessler, C. & Manta, V. (1990) *Gene* **92**, 1–250.
- 3 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	$5 \times 1$ ml ( $10 \times$ conc. solution)	11 417 959 001
SuRE/Cut Buffer B	Restriction enzyme incubation	$5 \times 1$ ml ( $10 \times$ conc. solution)	11 417 967 001
SuRE/Cut Buffer H	Restriction enzyme incubation	$5 \times 1$ ml ( $10 \times$ 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	$5 \times 1$ ml ( $10 \times$ conc. solution)	11 417 983 001
Water, PCR Grade	Specially purified, double-distilled.	100 ml (4 vials of 25 ml)	03 315 843 001
	deionized, and autoclaved	25 ml (25 vials of 1 ml)	03 315 932 001
	aatoolavou	25 ml 25 ml (1 vial of 25 ml)	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.
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#### **Commonly used bacterial strains**

Strain	Genotype
BL21	E. coli B F <sup>-</sup> dcm ompT hsdS(r <sub>B</sub> - m <sub>B</sub> -) gal (Studier, F.W. et al (1986) J. Mol. Biol., <b>189</b> , 113.)
C600 <sup>e</sup>	supE44 hsdR2 thi-1 thr-1 leuB6 lacY1 tonA21; (Hanahan, D. (1983) J. Mol. Biol. <b>166</b> , 557.)
DH5α	supE44 Δ(lacU169 (φ80dlacZΔM15) hsaR17 recA1 endA1 gyrA96 thi-1 relA1; (Hanahan, D. (1983) J. Mol. Biol. <b>166</b> , 557.)
HB101	supE44 hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1; (Hanahan, D., (1983) J. Mol. Biol. <b>166</b> , 557.)
JM108	recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi $\Delta$ (lac-proAB); (Yanisch- Perron, C. et al., (1985) Gene <b>33</b> , 103.)
JM109	recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi $\Delta$ (lac-proAB) F[traD36proAB <sup>+</sup> , lacl <sup>q</sup> lacZ $\Delta$ M15]; (Yanisch- Perron, C. et al., (1985) Gene <b>33</b> , 103.)
JM110	rpsL ( $St^F$ ) thr leu thi-l lacY galK galT ara tonA tsx dam dcm supE44 $\Delta$ (lac-proAB) $F$ [traD36proAB $^+$ , lac $^F$ lac $Z\Delta$ M15]; (Yanisch- Perron, C. et al., (1985) Gene <b>33</b> , 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., <b>16</b> , 118.)
SURE <sup>r</sup>	recB recJ sbc C201 uvrC umuC::Tn5(karf) lac , Δ(hsdRMS) endA1 gyrA96 thi relA1 supE44 F'[proAB <sup>+</sup> lacl <sup>q</sup> lacZΔM15 Tn10 (tet <sup>t</sup> ); (Greener, A. (1990) Stratagies, <b>3</b> , 5.)
TG1	supE hsd Δ5 thi Δ(lac-proAB) F[traD36proAB <sup>+</sup> , lacl <sup>q</sup> lacZΔM15]; (Gibson, T.J. (1984) PhD Theses. Cambridge University, U.K.)
XL1-Blue <sup>r</sup>	supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac F'[proAB <sup>+</sup> , lacl <sup>q</sup> lacZΔM15 Tn10 (tet <sup>f)</sup> ]; (Bullock et al., (1987) BioTechniques, 5, 376.)

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