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Restriction Endonuclease *Kpn* I

From *Klebsiella pneumoniae OK8*

Cat. No. 10 899 186 001 5000 units (10 U/µl)
Cat. No. 10 742 953 001 10 000 units, high concentration (40 U/µl)

Version 21
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.
Product is shipped on dry ice.

Sequence specificity *Kpn* I recognizes the sequence GGTAC/C and generates fragments with 3´-cohesive termini (1).

Compatible ends *Kpn* I has no compatible ends to other known restriction enzymes.

Isoschizomers *Kpn* I is an isoschizomer of *Asp* 718 which generates fragments with 5' cohesive termini.

Methylation sensitivity *Kpn* I is not inhibited by 5-methylcytosine at either or both C-residues or by 6-methyladenine.

Storage buffer 20 mM Tris-HCl, 50 mM NaCl, 0.1 mM EDTA, 10 mM 2-Mercaptoethanol, 0.01% Polydocanol, 50% Glycerol (v/v), pH approx. 7.5 (at 4°C).

Suppl. Incubation buffer (10×) 100 mM Tris-HCl, 100 mM $MgCl_2$, 10 mM Dithioerythritol, pH 7.5 (at 37°C), (= SuRE/Cut Buffer **L**).

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

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

Important Note For restriction digestion with *Kpn* I, the supplied SuRE/Cut buffer **L** requires **addition of bovine serum albumin** (100 µg/ml final concentration).

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 25 µl SuRE/Cut buffer **L** including 100 µg/ml BSA (final concentration).

Typical experiment

Component	Final concentration
DNA	1 µg
10 × SuRE/Cut Buffer **L**	2.5 µl
BSA (Cat. No. 711 454)	100 µg/ml
Sterile redist. water	Up to a total volume of 25 µl
Restriction enzyme	1 unit

Incubate at **37°C** for 1 h.

Heat Inactivation Up to 3 Units *Kpn* I/µg can be heat-inactivated by 15 min incubation at 65°C, higher concentrations can no more be completely inactivated under these conditions

Number of cleavage sites on different DNAs (2):

λ	Ad2	SV40	Φ X174	M13mp7	pBR322	pBR328	pUC18
2	8	1	0	0	0	0	1

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 *Kpn* I fragments obtained by complete digestion of 1 µg λ × *Eco* RI fragments 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) resulting in >95 % recovery of 1 µg λDNA × *Eco R*I fragments. Subsequent re-cutting with *Kpn* I yields > 95% of the typical pattern of λDNA × *Eco R*I × *Kpn* I fragments.

Troubleshooting A critical component is the DNA substrate.
Many compounds used in the isolation of DNA such as 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.

Star activity *Kpn* I may exhibit star activity under non-optimal conditions. The recognition specificity of *Kpn* I is altered by addition of increasing amounts of hydrophobic reagents and glycerol to the incubation mixture (2).

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 L with excess of *Kpn* 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 *Kpn* 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 Tomassini, J. et al. (1978) *Nucleic Acids Res.* **5,** 4055.
2 Kessler, C. & Manta, V. (1990) *Gene* **92**, 1–248.
3 Rebase The Restriction Enzyme Database: http://rebase.neb.com

Ordering Information

Product	Application	Packsize	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)	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

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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*$^+$, *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.)

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